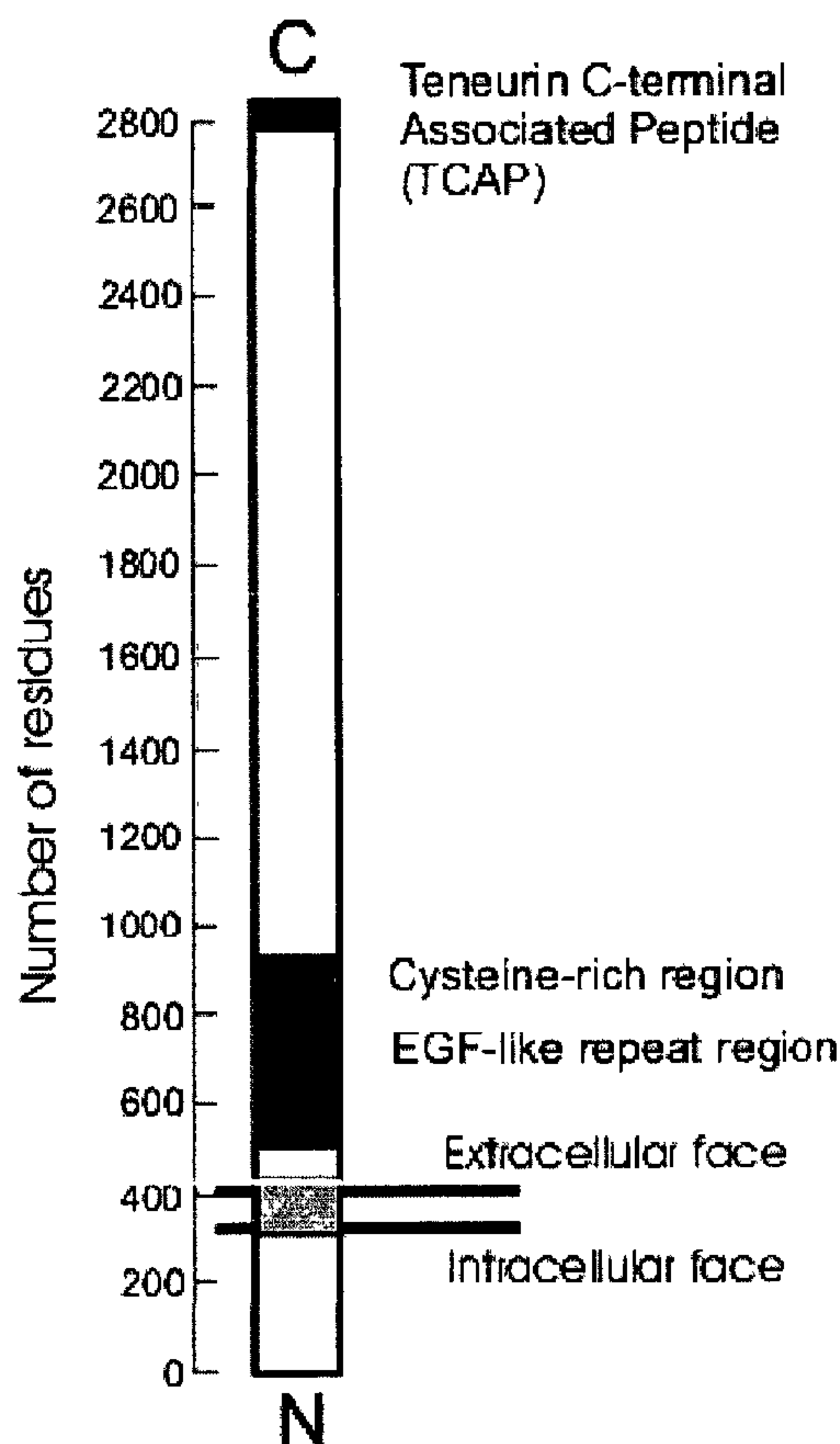




(86) Date de dépôt PCT/PCT Filing Date: 2003/05/02
 (87) Date publication PCT/PCT Publication Date: 2003/11/13
 (85) Entrée phase nationale/National Entry: 2004/10/15
 (86) N° demande PCT/PCT Application No.: CA 2003/000622
 (87) N° publication PCT/PCT Publication No.: 2003/093305
 (30) Priorités/Priorities: 2002/05/02 (60/376,879) US;
 2002/05/03 (60/377,231) US; 2002/11/06 (60/424,016) US

(51) Cl.Int.⁷/Int.Cl.⁷ C07K 14/435, A61K 38/17, C07K 16/18
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(54) Titre : PEPTIDES ASSOCIES AU TERMINAL C DE TENEURINES (TCAP) ET LEURS PROCEDES D'UTILISATION
 (54) Title: TENEURIN C-TERMINAL ASSOCIATED PEPTIDES (TCAP) AND USES THEREOF



(57) **Abrégé/Abstract:**

The invention provides a novel family of biologically active neuropeptides and the nucleic acid molecules coding for same. The peptides are derived for the C-terminus of the teneurin family peptides (Ten M1-4). These novel peptides, referred to as teneurin C-terminal associated peptides (TCAPs) are active in neuronal communication and are implicated in a number of neuropathologies. They are particularly useful in modulating stress responses and anxiety and in the treatment of cancer.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 November 2003 (13.11.2003)

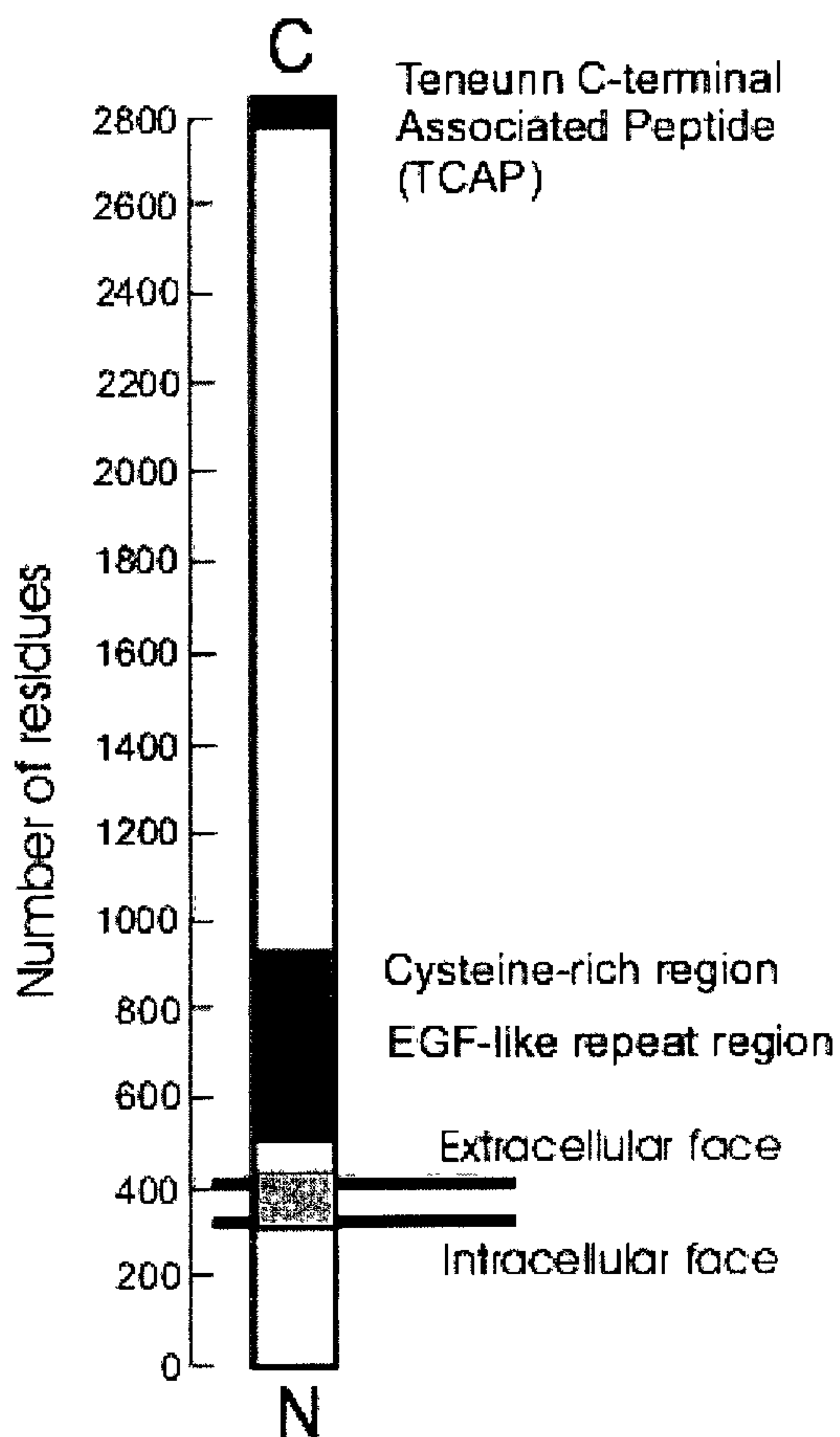
PCT

(10) International Publication Number
WO 2003/093305 A3

- (51) International Patent Classification⁷: **C07K 14/435**,
A61K 38/17, C07K 16/18
- (21) International Application Number:
PCT/CA2003/000622
- (22) International Filing Date: 2 May 2003 (02.05.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/376,879 2 May 2002 (02.05.2002) US
60/377,231 3 May 2002 (03.05.2002) US
60/424,016 6 November 2002 (06.11.2002) US
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- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: TENEURIN C-TERMINAL ASSOCIATED PEPTIDES (TCAP) AND USES THEREOF



(57) Abstract: The invention provides a novel family of biologically active neuropeptides and the nucleic acid molecules coding for same. The peptides are derived for the C-terminus of the teneurin family peptides (Ten M1-4). These novel peptides, referred to as teneurin C-terminal associated peptides (TCAPs) are active in neuronal communication and are implicated in a number of neuropathologies. They are particularly useful in modulating stress responses and anxiety and in the treatment of cancer.

WO 2003/093305 A3

WO 2003/093305 A3

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
5 February 2004

Published:

— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE: Teneurin C-Terminal Associated Peptides (TCAP) and Methods
and Uses Thereof

RELATED APPLICATIONS

This application claims the benefit and priority of United States
5 provisional patent application number, US 60/377,231, Filed May 3, 2002,
entitled "Teneurin C-Terminal Associated Peptides (TCAP)" and US
60/42,4016, filed November 6, 2002, entitled "Method for Modulating Stress
using Teneurin C-Terminal Associated Peptide-1(TCAP-1)". This application
also claims priority from United States provisional patent application number,
10 US 60/ 376,879, filed May 2, 2002, entitled, " Immortalized Hypothalamic
Neuronal Cell Lines ". All of these references are incorporated in their entirety
by reference.

FIELD OF THE INVENTION

The invention relates to a novel family of peptides associated with the
15 c-terminal region of the teneurin molecule, to a nucleic acid molecule
encoding said peptides and to methods and uses therefore.

BACKGROUND OF THE INVENTION

The aetiology of any neuropathology is a complex interplay of genetic,
physiological and environmental factors. Effective treatment of these
20 conditions will ultimately depend upon the understanding of the cognate
genes and their products. In recent years, it has become apparent that large
families of related genes are responsible for the regulation of
neuropathologies involving anxiogenic peptides. The identification and
characterization of these gene families and how they interact is an essential
25 step towards ultimately effectively treating the pathology. The aberrant
regulation of neuronal growth can manifest as a variety of pathological
conditions depending upon the age. Deficits in neuronal growth in foetal or
neonatal animals can cause such diseases as learning deficits, mental
retardation, autism, or schizophrenia. At later ages in juvenile individuals it
30 may manifest as affective disorders such as panic disorder, depression,
anorexia nervosa, obsessive-compulsive disorder later in adults. In adults

-2 -

such neuronal growth problems could lead to neurodegenerative illnesses such as Alzheimer's Disease or Parkinson's Disease.

The onset of mood disorders, such as depression or post traumatic stress disorder, involve the altered function of multiple loci in the brain that regulate emotionality, memory and motivation (Manji et al., 2001; Drevets, 2001; Nestler et al., 2002). However, many of the cellular signaling molecules that mediate communication within and between these regions are unknown, leading to an incomplete understanding of the origin of such disorders.

Many neuropeptides show the presence of three or four paralogous structures as evidenced by the neuropeptide Y (NPY) (Larhammar, 1996a,b), proopiomelanocortin (POMC) (Danielson, 2000) and recently, the corticotropin releasing factor (CRF) family (Vale et al., 1981, Vaughan et al., 1995; Lovejoy and Balment, 1999; Lewis et al., 2001 Reyes et al., 2001; Hsu and Hseuh, 2001).

A family of neuronal cell surface proteins has been identified that are predominantly expressed in the nervous system. These proteins have been named teneurins (Rubin et al, *Developmental Biology* 216, 195-209 (1999)). Four basic teneurins have been identified Ten M1, Ten M2, Ten M3, and Ten M4. The Ten-M or Odz proteins were originally discovered in *Drosophila* (Levine et al., 1994; Baumgartner et al., 1994) and are presently the only known example of a pair-rule gene that is not a transcription factor. The Ten-M gene is initially activated during the blastoderm stage, then down regulated before being expressed at later stages. The highest levels of Ten-M occur in the central nervous system where the protein occurs preferentially on the surface of axons (Levine et al., 1994; Levine et al, 1997). Mutations of the Ten-M/Odz gene result in embryonic lethality (Baumgartner et al., 1994; Levine et al., 1994).

Four Ten-M paralogous genes, called Teneurins, exist in vertebrates and encode a Type II transmembrane protein where the carboxy terminus of the protein is displayed on the extracellular face of the cell (Oohashi et al., 1999). The teneurin proteins are about 2800 amino acids long. There is a short stretch of hydrophobic residues at 300 to 400 amino acids after the

-3 -

amino terminus that appear to act as the membrane spanning site. In the cytoplasmic N-terminal portion, is a conserved proline-rich SH3-binding site indicating a potential site where by they bind other proteins. Evidence suggests that the protein may be cleaved from the membrane at a Furin-like cleavage motif (RERR) located around residue 528 in teneurin 2 (Rubin et al., 1999). However, this motif is not present in the other paralogues and therefore a soluble version of the protein may not occur for all paralogues. There are a series of cysteine-rich EGF-like repeats carboxy terminal to this. Homodimerization occurs between Ten M1 forms via interaction between EGF-like modules 2 and 5 (Oohashi et al., 1999).

The ten-m gene appears to be upregulated by stressors. Wang et al (1998) showed that a ten-M like transcript, named DOC4 (downstream of chop) in mammalian cells was upregulated by the transcription factor GADD153/CHOP. This transcription factor is induced by several types of cellular stressors including UV light, alkylating agents or conditions triggering endoplasmic reticulum (ER) stress responses, such as, deprivation of oxygen, glucose or amino acids, or interference of calcium flux across the ER membrane (Zinszner et al, 1998). GADD153 is a small nuclear protein that dimerizes with members of the C/EBP family of transcription factors (Ron and Habener, 1992). It does not appear to homodimerize. GADD153 undergoes a stressor inducible phosphorylation by a p38-type MAP kinase which also enhances the transcriptional activation of GADD153 (Wang et al., 1996). High expressions of GADD153 will lead to cell cycle arrest (Zhan et al. 1994). These studies suggest that the teneurin gene may play a significant role in the regulation of the stress response of neurons and other cells.

Overexpression of teneurin 2 into the mouse neuroblastoma cells (Nb2a) augmented the amount of neurite outgrowth and a tendency to enlarge the growth cones. The number of filamentous actin-containing filopodia was also enhanced in the teneurin 2 overexpressing cells (Rubin et al., 1999). The expression of the teneurin genes have been examined in embryonic zebrafish (Mieda et al, 1999), chicken (Rubin et al., 1999) and mouse (Ben-Zur et al., 2000) although their expression patterns have not been finely resolved. The

-4 -

transcripts are found in a number of peripheral tissues but are found predominantly in the central nervous system. In the embryonic chicken brain, teneurin 1 and 2 are expressed in the retina, telencephalon, the optic tectum and the diencephalons. The mRNA for teneurin 1 was found mainly in the
5 intermediate zone of the dorsal thalamus whereas teneurin 2 was found in the intermediate zone of the thalamus (Rubin et al., 1999). In zebrafish, teneurin 4 is faintly expressed throughout gastrulation, although there is no teneurin 3 expression. Teneurin 3 expression begins at the notochord and the somite around the tailbud stage. In later stages (14 h post fertilization), teneurin 3 is
10 expressed in the somites, notochord and brain while teneurin 4 expression was confined to the brain. Teneurin 3 becomes defined within the optic vesicles and region covering the caudal diencephalons and mesencephalon with the expression strongest in the anterior mesencephalon. Teneurin 4 has its strongest expression toward the midbrain hindbrain border. By 23 h post
15 fertilization, teneurin 3 is expressed in the dorsal part of the tectal primordium and the ventral midbrain while teneurin 4 is expressed in the ventral primordium (Mieda et al., 1999).

Neuropathological conditions tend to be complex and not very well understood. As such, there is a need to better understand the mechanisms
20 involved and to develop a method of diagnosis and treatment of said conditions. There is also a need for the identification and design of therapeutic compounds for said conditions.

SUMMARY OF THE INVENTION

25 The present invention provides a teneurin c-terminal associated peptide (TCAP), existing as a 40 - 41-residue sequence on the c-terminal exon of Ten- M 1, 2, 3, or 4 that is correspondingly named TCAP 1, 2, 3, and 4. In another embodiment, the invention provides a peptide that has the amino acid sequence consisting of a 40- or 41 amino acid sequence located at the c-
30 terminus of the teneurin 1-4 peptides, to analogs, species homologues, derivatives, variants, allelic variants, to sequences having substantial sequence identity thereto and to obvious chemical equivalents thereto. In

-5 -

another embodiment the TCAP peptides of the invention can further include an amidation signal sequence at the carboxy terminus (hereinafter referred to as "preTCAP"). Such amidation signal amino acid sequence can include but is not limited to GKR and GRR. The invention also provides fusion proteins
5 comprising the TCAP peptides noted above, to labeled TCAP Peptides and to peptides comprising flanking amino acid sequence of 1-10 amino acids.

In one embodiment, the invention provides a TCAP peptide that has neuronal communication activity. In another embodiment the invention provides a TCAP peptide, an analog, derivative, variant, homolog that has
10 similar activity. In one embodiment, the activity is neuronal communication. In another embodiment it is inhibition of cell proliferation, In yet another embodiment it is modulation of a stress response.

In one embodiment the TCAP sequence is a rainbow trout, zebrafish, human, mouse, *G. gallus*, or *D. melanogaster* TCAP. In another embodiment,
15 the TCAP sequence comprises or consists of SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103
In yet another embodiment, the TCAP is a mouse or human TCAP. In one embodiment the TCAP has one of the sequence selected from the group consisting of SEQ. ID. NOS: 69, 70, 77, 78, 85, 86, 93, 94 (human) or SEQ.
20 ID. NOS: 37, 38, 45, 46, 53, 54, 61, 62, (mouse).

In one aspect, the invention provides a TCAP consisting of any one of the SEQ. ID. NOS. noted above and an amidation signal sequence at the carboxy terminus. Preferably the amidation signal sequence is selected from the group consisting of GRR or GKR, such as, 15, 16, 23, 24, 31, 32, 39, 40,
25 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96.

Another aspect of the invention relates to an isolated teneurin c-terminal associated peptide that has the amino acid sequence as shown in SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103; or a fragment, analog, homolog, derivative or mimetic
30 thereof. In a preferred embodiment, the TCAP peptides of the invention have angiogenic activity. The invention also encompasses an antibody that can bind a TCAP peptide of the invention.

-6 -

In another embodiment, the peptide of the invention is a TCAP mouse peptide having the amino acid sequence of: SEQ. ID. NOS: 37, 38, 45, 46, 53, 54, 61, 62.

In another embodiment, the peptide of the invention is a TCAP human peptide having the amino acid sequence of SEQ. ID. NOS: 69, 70, 77, 78, 85, 86, 93, or 94.

In another embodiment the peptides TCAP human and mouse peptides have an amidation signal sequence at the C-terminus.

In another embodiment, the peptide of the invention is a TCAP-1 and has the amino acid sequence of SEQ. ID. NOS.: 37,38, 69 or 70.

In another embodiment, the peptide of the invention is a TCAP-2 and has the amino acid sequence of SEQ. ID. NOS.: 46, 47, 77, or 78.

In another embodiment, the peptide of the invention is a TCAP-3 and has the following amino acid sequence motif:

15 QLLSXaa₁Xaa₂KVXaa₃GYDGYVLSXaa₄EQYPELADSANNXaa₅QFL
RQSEI (SEQ. ID. NO:135) ,

where Xaa₁ is G, S, or A; Xaa₂ is G or R; Xaa₃ is L or Q; Xaa₄ and Xaa₅ are independently V or I. In one embodiment, the TCAP-3 is a human or mouse TCAP- 3. In another embodiment, the TCAP- 3 has SEQ. ID. NO: 85, 86, 53, 20 or 54. In another embodiment, the TCAP 3 sequence is SEQ. ID. NO.: 13, 14, 21 or 22.

In another embodiment, the peptide of the invention is a TCAP-4 and has the amino acid sequence SEQ. ID. NOS.: 29, 30, 61, 62, 93, or 94.

In another embodiment the peptides TCAP 1 to TCAP 4 have an amidation signal sequence at the C-terminus.

In yet another embodiment, the present invention provides as isolated nucleic acid molecule encoding a teneurin c-terminal associated peptide (TCAP) of the invention, as noted herein. In yet another embodiment, the isolated nucleic acid molecule of the invention consists of:

30 (a) a nucleic acid sequence as shown in SEQ.ID.NOS.: 18-20, 25-28, 33-36, 41-44, 49-52, 57-60, 65-68, 73-76, 81-84, 89-92, 97-100 or that wherein T can also be U or that encodes a peptide having an amino acid

-7 -

sequence selected from the group consisting of : SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or that further has an amidation signal sequence (preferably GKR or GRR), at the carboxy terminus of said peptides, such as 15, 16, 23, 24, 31, 5 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96;

(b) a nucleic acid sequence that is complimentary to a nucleic acid sequence of (a) or (b);

(c) a nucleic acid sequence that has substantial sequence homology to a nucleic acid sequence of (a), or (b);

10 (d) a nucleic acid sequence that is an analog of a nucleic acid sequence of (a), (b), or (c); or

(e) a nucleic acid sequence that hybridizes to a nucleic acid sequence of (a), (b), (c), or (d) under stringent hybridization conditions.

15 In a preferred embodiment the nucleic acid molecules of the invention encode teneurin c-terminal associated peptide that has anxiogenic activity.

The invention also encompasses antisense oligonucleotides complimentary to a nucleic acid sequence of the invention as well as expression vectors comprising a nucleic acid molecule of the invention and host cells transformed with the aforementioned expression vectors.

20 A further aspect of the invention relates to a method of identifying substances which can bind with a teneurin c-terminal associated peptide, comprising the steps of incubating a teneurin c-terminal associated peptide and a test substance, under conditions which allow for formation of a complex between the teneurin c-terminal associated peptide and the test substance, 25 and assaying for complexes of the teneurin c-terminal associated peptide and the test substance, for free substance or for non complexed teneurin c-terminal associated peptide, wherein the presence of complexes indicates that the test substance is capable of binding a teneurin c-terminal associated peptide.

30 The invention also provides a method of identifying a compound that affects the regulation of neuronal growth comprising incubating a test compound with a teneurin c-terminal associated peptide or a nucleic acid

-8 -

encoding a teneurin c-terminal associated peptide; and determining an amount of teneurin c-terminal associated peptide protein activity or expression and comparing with a control, wherein a change in the TCAP peptide activity or expression as compared to the control indicates that the test compound has
5 an effect on the regulation of neuronal growth.

The invention also provides a method of inhibiting cell proliferation comprising administering to a cell, an effective amount of teneurin c-terminal associated peptide that inhibits cell proliferation. In a preferred embodiment, the inhibited cells are selected from the group consisting of neuronal or
10 fibroblast cells.

Another aspect of the invention relates to a method of detecting a condition associated with the aberrant regulation of neuronal growth comprising assaying a sample for a nucleic acid molecule encoding a teneurin c-terminal associated peptide or a fragment thereof or a teneurin c-terminal
15 associated peptide or a fragment thereof.

The invention also relates to a method of treating a condition associated with the aberrant regulation of neuronal growth, for instance cancer, comprising administering to a cell or animal in need thereof, an effective amount of teneurin c-terminal associated peptide or an agent that modulates
20 teneurin c-terminal associated peptide expression and/or activity.

The teneurin-1 mRNA containing the TCAP-1 sequence is expressed in regions of the forebrain and limbic system regulating stress responses and anxiety. TCAP signals through a specific cAMP-dependent G-protein-coupled receptor to modify cell cycle and proliferation in immortalized neurons.
25 Administration of synthetic TCAP-1 into the lateral ventricle or amygdala of rats normalized the acoustic startle response. These peptides, therefore, appear to be an integral part of the neural stress response and likely play a role in the aetiology of some psychiatric illnesses.

In another embodiment, the invention provides a method of modulating
30 the stress response in an animal, preferably in a mammal, preferably a human, by administering to said animal an effective amount of TCAP, preferably TCAP-1 peptide, a nucleic acid molecule coding for said TCAP

-9 -

peptide in a form that can express said peptide *in situ* or an antagonist or agonist of TCAP expression or activity, to modulate the stress response in said animal. In one embodiment the stress response is an anxiety response.

In another embodiment, the invention provides a method for
5 normalizing the stress or anxiety response in an animal. In another embodiment, the invention provides a method for inducing an anxiogenic response in a low anxiety animal and for inducing an anxiolytic effect in a high anxiety animal.

In another embodiment, the invention provides a method modulating
10 the stress response in an animal by modulating the effect of TCAP expression in an animal by administering to said animal a modulator of said TCAP expression or activity. In one embodiment said modulator is an inhibitor of TCAP expression and/or activity, in another embodiment, said modulator is an antagonist of TCAP expression or activity. In one embodiment said TCAP is
15 TCAP-1.

In yet another embodiment, said invention provides a method of
diagnosing an animal with high, normal or low stress response condition by administering to said animal a TCAP, such as TCAP-1 and monitoring whether
it has an anxiolytic, anxiogenic or neutral effect on a stress response of the
20 animal.

Other aspects of the invention relate to methods of inducing an
anxiogenic response in a subject, methods of inhibiting damages caused by physiological stresses and methods of inhibiting cell death, each comprising
administering to a subject an effective amount of teneurin c-terminal
25 associated peptide for affecting the desired result.

Other features and advantages of the present invention will become
apparent from the following detailed description. It should be understood,
however, that the detailed description and the specific examples while
indicating preferred embodiments of the invention are given by way of
30 illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

-10 -

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 shows a putative 3' exon of the rainbow trout Teneurin 3 gene [SEQ. ID. NO: 2] with an intron region [SEQ. ID. NO: 1] (1490 bp). The exon/intron border as established by sequence comparison with the human ten M1 gene (LocusLink ID# 10178) shown in the genome database. The intron placement was subsequently confirmed by PCR. The exon encodes the carboxy terminal 251 residues of the protein SEQ. ID. NO: 3. Cleavage signals are indicated in the bolded grey regions. The Terminal GKR motif usually signifies a post translation amidation signal. The teneurin-associated c-terminal peptide (TCAP) is shown by the sequence between amino acids 208 and 248 inclusive [SEQ. ID. NOS: 13 and 14].

Figure 2 shows the alignment of the amino acid sequences encoded by the terminal exon of the rainbow trout (*O. mykiss*) SEQ. ID.NO: 3, zebrafish (*R. danio*) SEQ. ID.NO: 12, mouse (*M. musculus*) SEQ. ID.NO: 6 and human (*H. sapiens*) SEQ. ID.NO: 10 genes. All possess an additional serine insertion in position 58. All show a high sequence similarity with about 94% between trout and zebrafish, 83% between rainbow trout and mouse, and 83% between rainbow trout and human. Within the TCAP portion itself, rainbow trout SEQ. ID. NO: 13 or 14 shares 90% sequence identity with zebrafish SEQ. ID. NO: 21 or 22, 90% sequence identity with mouse SEQ. ID. NO:53 or 54, and 88% with human SEQ. ID. NO. 85 or 86. The preTCAP sequences that include the amidation signal are SEQ. ID. NOS: 15 - 16 (Rainbow Trout), 23 - 24 (zebrafish), 55 - 56 (mouse) and 87 -88 (human).

Figure 3 shows the alignment of the amino acid sequences encoded by the terminal exon of the mouse teneurin 1, 2, 3 and 4) SEQ. ID. NOS: 4, 5, 6, 7 genes. The highest level of sequence similarity occurs among the sequences encoding the TCAP portion of the protein. TCAP-1 SEQ. ID. NO: 37 or 38 is 68% identical to TCAP-2 SEQ. ID. NO. 45 or 46, 76% identical to TCAP-3 SEQ. ID. NO. 53 or 54, and 85% identical to TCAP-4 SEQ. ID. NO. 61 or 62. TCAP-2 is 75% identical with TCAP-3, and 68% identical with

-11 -

TCAP-4. TCAP-3 possesses 71 % identity with TCAP-4. Teneurin 3 possesses a dibasic cleavage site at the amino terminus of TCAP-3 whereas 1, 2 and 4 all possess monobasic sites suggesting that the cleaved peptide is 40 residues in TCAP-3 but 41 residues in TCAP-1, 2 and 4. However, in one 5 embodiment, both the 41 and 40 amino acid residue TCAP has activity.

Figure 4 shows the alignment of amino acid sequences encoded by the last exon of the human Teneurin 1, 2, 3 and 4 proteins SEQ. ID. NOS: 8, 9, 10, 11. Like the mouse sequence, the highest degree of sequence similarity occurs in the TCAP portion of the exon. TCAP-3 possesses a dibasic leaved 10 signal whereas the others possess a monobasic site. The greatest variable region occurs with the first 70- 80 residues of the exon. Within the TCAP portion itself, TCAP-1 SEQ. ID. NO: 69 or 70 shares 73% identity with TCAP-2 SEQ. ID. NO: 77 or 78, 83% identity with TCAP-3 SEQ. ID. NO: 85 or 86 and 88% identity with TCAP-4 SEQ. ID. NO. 93 or 94. TCAP-2 has 76% 15 identity with TCAP-3 and 71% identity with TCAP-4. TCAP-3 has 76% identity with TCAP-4.

Figure 5 shows the nucleotide coding sequence of the preTCAP sequences for Human (SEQ. ID. NOS: 76, 84, 92, and 100) and Mouse (SEQ. ID. NOS. 44, 52, 60 and 68) preTCAP-1 to 4, Zebrafish preTCAP-3 and 4 20 (SEQ. ID. NOS: 28 and 36), and Rainbow Trout preTCAP-3 (SEQ. ID. NO. 20) with stop codon. The coding region of the corresponding mature TCAP peptides would lack the terminal amidation and stop codon coding sequence (e.g. the last 12 nucleotide bases shown for each sequence). The sequences shown code for the 44 amino acid residue preTCAP sequence with stop 25 codon. However, the 43 amino acid TCAP coding sequence is identical except with the first three nucleotides absent.

Figure 6A is a schematic representation of the functional domains within the Teneurin protein. Figure 6B is a schematic view of the exons on human teneurin 1 and an exploded view of the location of the C-terminal exon 30 and location of TCAP thereon. A conserved prohormone convertase-like cleavage motif is shown as grey boxes. It illustrates the structure of Teneurin

-12 -

C-terminal Associated Peptides and their location on the teneurin protein and gene.

Figure 7A shows the alignment of the human, mouse, rat, chicken, rainbow trout, zebrafish and drosopholia TCAP sequences SEQ. ID.NOS: 69, 5 78, 85, 94, 37, 46, 53, 66, 78, 101, 136, 13, 21, 30 and 103 and 7B shows the alignment of the TCAP sequences from mammals birds insects and nematodes Fig. 7B SEQ. ID. NOS: 37, 101 (without the Q at the N-terminus), 69, 61, 93, 53, 85, 13, 21, 77, 30, and 103. In figure 7B, non homologous amino acid substitutions are shaded in light grey. Homologous residues are 10 shaded in dark grey.

Figure 8 shows the alignment of the amino acid sequences of the human CRF family SEQ. ID. NOS: 104 – 107 with those of the human TCAP family SEQ. ID.NOS: 70, 78, 85, 94. Although overall sequence identity is only about 20-25 %, many of the other substitutions reflect potential single base 15 codon changes such as proline to serine, leucine or threonine, or conservative amino acid substitutions such as leucine to valine or isoleucine, aspartic acid to glutamic acid and asparagines to glutamine.

Figure 9 is a comparison of the sequence identity among CRF family members to that of the identity among TCAP members. The TCAP family 20 members show a much greater sequence identity of 68% compared to the CRF family members of 34% between CRF and U3 and U2, 43% between CRF and urocortin, and 21% between urocortin 1 and 3.

Figure 10 shows a secondary structure prediction of TCAP (Rainbow Trout TCAP-3) and comparison with CRF-like peptides. Figure 10 A is a 25 Grantham Polarity Prediction and Figure 10B is a Kyte-Doolittle Hydrophobicity Prediction. TCAP shows a highly similar polarity profile, but appears to possess higher levels of total hydrophobicity in the amino terminus.

Figure 11 shows the alignment of amino acid sequences of 30 representations of TCAP peptides with the insect diuretic peptides and CRF superfamily SEQ. ID. NOS: 13, 22, 104, 107-110. The entire superfamily can

-13 -

be divided into three general regions encompassing an amino terminal portion, a midsection and a carboxy terminal portion. All peptides can be aligned by the presence of conserved motifs within each of the separate sections

5 Figure 12 illustrates expression of Teneurins in mouse brain and cell lines NLT, Gn11, and Nero2a . PCR-amplified products corresponding to Teneurin 1 to 4 were found in whole brain and cell lines. TenM1,2 and 4 were found in whole brain and in the immortalized GnRH-expressing neuronal line, Gn11. Only Teneurin 2 and 4 were found in another GnRH-expressing cell,
10 NLT, however, all four forms were found in the Neuro2a neuroblastoma cell line. The bands on top indicate positive signals for the Teneurin transcripts. The bands at the bottom show a positive signal for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) to indicate the viability of the RNA. A 100-bp DNA ladder is shown at the left of all PCR gels.

15 Figure 13 is a bar graph illustrating the inhibition of cell proliferation in Gn11 neuronal cells by 10^{-6} M TCAP (Rainbow Trout TCAP-3) at 48 hours (Figure 13 A) and at 72 hours (Figure 13B).

Figure 14 is a bar graph illustrating the inhibition of cell proliferation in TGR1 (wildtype) fibroblast cells.

20 Figure 15 is a bar graph illustrating the inhibition of cell proliferation in HO16 (c-myc constitutively expressed cells) (14B) by 10^{-6} M TCAP (Rainbow Trout TCAP-3) at 48 hours).

Figures 16A and 16B are bar graphs illustrating the inhibition of cAMP(16A) and cGMP (16B) accumulation in Gn11 cells by rtTCAP-
25 3(Rainbow Trout TCAP-3). A. 10^{-6} M TCAP induced a significant ($p<0.01$) decrease in cAMP concentrations relative to the vehicle-treated cells. Replications: vehicle, n=10; urocortin, n=8; TCAP, n=11. B. 10^{-6} M TCAP induced a significant ($p<0.01$) decrease in cGMP accumulation in Gn11 cells. The same concentration of rat urocortin also induced a significant ($p<0.05$)
30 decrease in cGMP concentrations. Three replications were used for each of the treatment groups. Significance was assessed using a one-way analysis of

-14 -

variance with a Dunnett's post-hoc test. An a priori level of significance was established at $p=0.05$. The original data was transformed to show percent concentration relative to the vehicle-treated cells.

Figure 17 A-D illustrates TCAP(Rainbow Trout TCAP-3) cAMP regulation in Gn11 cells. 17A illustrates cAMP levels in Gn11 cells treated with 10^{-8} M TCAP or urocortin over 30 minutes. 17B illustrates cAMP levels in Gn11 cells in the presence of 10^{-4} M 3-isobutyl-1 methyl xanthine (IBMX), a phosphodiesterase inhibitor used to boost cAMP induced by treatment of 10^{-8} MTCAP or urocortin. 17C is a bar graph illustrating cAMP accumulation over 30 minutes in Gn11 cells by administration of various concentrations of TCAP or Urocortin in the presence of IBMX. 17D is a bar graph illustrating inhibition of 10^{-6} M forskolin- stimulated cAMP by 10^{-8} MTCAP or urocortin.

Figures 18A and 18B are linear graphs illustrating the effect of TCAP (Rainbow Trout TCAP-3) on the administration of self reward behaviour. The behaviour was indicated by number of bar presses per 30 seconds over a range of pleasurable stimulation (25 – 100Hz). Figure 18A: Baseline, TCAP peptide (1.0 μ l of 0.001mg/ml, left), post-injection (approx. 90 min.), 850uA. Figure 18B: Baseline, TCAP peptide ((1.0 μ l of 0.001mg/ml, right), postinjection (approx. 60 min.), 550uA. 100 nM TCAP induced a significant decrease in the rats desire to self-administer reward by neural impulse.

Figure 19 A schematic cellular model for TCAP regulation. A. A stressor in the form of a physiological condition such as low oxygen or pH changes, or an anxiogenic ligand triggers metabolic activation of the cell. B. This causes an upregulation of the Teneurin protein and its cleaving enzyme. C. The enzyme liberates TCAP from Teneurin where it acts in an autocrine and paracrine manner to inhibit cAMP and cGMP production via a G protein coupled receptor.

Figure 20 illustrates the distribution of TCAP-1 mRNA in rat brain nuclei as explained in Example 9.

Figure 21 are bar graphs illustrating the chronic human TCAP-1 response in rats that were (A) vehicle treated ICV injected, (B)TCAP-1 ICV injected as described in Example 10 herein.

-15 -

Figure 22 are graphs illustrating the mean baseline startle response of all animals in Example 10. Figure 22A is the average startle response at day 1 after TCAP injection and Figure 22B is the average startle at the end of the chronic TCAP study, Figure 22C is the average startle response following
5 TCAP-1.

Figure 23 is the interaction bar plot for treatment with TCAP-1 at various doses for both high and low anxiety response animals as discussed in Example 11 herein.

Figure 24 is the plot of the effect of TCAP-1 amygdala –injected on the
10 startle response of rats as discussed in Example 11 herein.

Figure 25 illustrates activity of TCAP on immortalized neurons. (A) cAMP accumulation in Gn11 cells. 1nM TCAP increased cAMP ($p<0.05$) whereas 100 nM TCAP decreased ($p<0.05$) cAMP. An intermediate concentration (10nM) was without effect. (B) Action of CRF-R1 antagonist on
15 cAMP accumulation. A 1 nM mouse TCAP-1, or mouse urocortin increased cAMP accumulation in Gn11 cells. The CRF R1 receptor antagonist PD171729 abolished the action of urocortin on these cells ($p<0.01$) but had no effect on TCAP-mediated cAMP accumulation. (C) Protein assays. Concentrations of 1 to 100 nM TCAP stimulated protein synthesis in Gn11
20 cells. (D) MTT Assay. 1 nM of mouse TCAP-1 increased MTT activity ($p<0.05$) in Gn11 cells after 48 hours. In contrast, 100 nM of mouse TCAP-1 decreased ($p<0.05$) MTT activity over the same time period. E. DNA Content Analysis. mouse TCAP-1 reduced the incidence of G1 phase at the lowest concentration of 1 nM, however, increased the number of cells in G1 phase at
25 the highest dose of 100 nM. The level of significance was determined using a one-way ANOVA for A, B and E, and a two-way ANOVA for C and D.

Figure 26 illustrates the functional cAMP response of murine hypothalamic immortalized cell lines to TCAP(rainbow trout TCAP-3) peptide stimulation.

30

DETAILED DESCRIPTION OF THE INVENTION

-16 -

The inventors have identified a novel peptide sequence which exists as part of a larger protein previously identified as the Ten M proteins or Teneurins. The novel peptides are referred to as teneurin C terminal peptides or TCAP. The genomes or gene transcripts of several vertebrate and
5 invertebrate species were screened by homologous probe hybridization or by PCR. Sequence data from genome sequencing programs allowed the identification of a complement of four paralogous peptides from this family in humans and mice, two paralogues in zebrafish, one in rainbow trout and *Drosophila* (SEQ ID NO:103). The synthetic TCAP peptide has neuronal
10 comunciation activity and has been shown to be a modulator of the stress response and anxiety in an animal. TCAP also modulates cell proliferation. In one embodiment, it can inhibit cell proliferation. In another embodiment, TCAP is a potent angiogenic peptide in rats and highly effective at inhibiting neuronal proliferation in unstressed cells and protecting cells from
15 physiological stresses. As such TCAP and/or modulators of TCAP can be used in the treatment of cancer and neuropathological conditions, including those related to neuronal communication, and/or cell proliferation, for instance, cancer, stress anxiety, food-related disorders, such as anorexia and/or obesity.

20 The TCAP sequence encodes a cleavable peptide 40 amino acids long flanked by PC7-like cleavage motifs on the amino terminus and an amidation motif on the carboxy terminus. Depending on the cleavage of the PC7-like cleavage site at the N-terminus, the resulting mature TCAP peptide is 40-41 amino acids in length. The TCAP sequence with the carboxy terminus
25 amidation motif is herein referred to as preTCAP. Orthologues in humans, mice, zebrafish and *Drosophila* as well as three additional paralogous sequences have been identified. A synthetic version of the rainbow trout peptide significantly increases the startle reflex and decreases self-administered brain stimulation in rats. These findings are consistent with the
30 actions of peptides known to induce anxiety in mammals and humans. The peptide is also potent at inhibiting the proliferation of unstressed neuronal and fibroblast cell cultures and inhibiting cell death in these cultures subjected to

-17 -

high pH stress. These findings indicate that TCAP plays a role in the developing and adult brain to modulate and protect neuronal growth and metabolism and therefore be implicated in a number of pathologies including schizophrenia, Parkinson's disease and other mental conditions. In the adult
5 brain the peptide may act to modulate the actions of anxiogenic stimuli and could play a role in depression, anorexia nervosa and other affective disorders.

The term "isolated" as used herein means "altered by the hand of man" from the natural state. If a composition or substance occurs in nature, the isolated
10 form has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated," as the term is employed herein. Thus, a polypeptide or polynucleotide produced and/or
15 contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" or an "isolated polynucleotide" are polypeptides or polynucleotides that have been purified, partially or substantially, from a recombinant host cell or from a native source. For example, a recombinantly produced version of TCAP peptides
20 and derivatives thereof can be substantially purified by methods known in the art, such as the one-step method described in Smith and Johnson, Gene 67:31-40 (1988).

Nucleic Acid Molecules of the Invention

25 The present invention provides an isolated nucleic acid molecule consisting of a sequence encoding a teneurin c-terminal associated peptide. This peptide is generally referred to as "TCAP" herein. The present invention also provides an isolated nucleic acid molecule encoding a TCAP peptide with a carboxy terminus amidation motif, said peptide herein referred to as
30 "preTCAP".

Isolated nucleic acids substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical

-18 -

precursors, or other chemicals when chemically synthesized are included in this invention.

In a preferred embodiment, the invention provides an isolated nucleic acid sequence comprising or consisting of:

- 5 (a) a nucleic acid sequence as shown in SEQ.ID.NOS.: 18-20, 25-28, 33-36, 41-44, 49-52, 57-60, 65-68, 73-76, 81-84, 89-92, 97-100 or that wherein T can also be U or that encodes a peptide having an amino acid sequence selected from the group consisting of : SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 10 103 or that further has an amidation signal sequence (preferably GKR or GRR), at the carboxy terminus of said peptides, such as 15, 16, 23, 24, 31, 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96;
- (b) a nucleic acid sequence that is complimentary to a nucleic acid sequence of (a);
- 15 (c) a nucleic acid sequence that has substantial sequence homology to a nucleic acid sequence of (a) or (b);
- (d) a nucleic acid sequence that is an analog of a nucleic acid sequence of (a), (b) or (c); or
- (e) a nucleic acid sequence that hybridizes to a nucleic acid 20 sequence of (a), (b), (c) or (d) under stringent hybridization conditions.
- (f) a nucleic acid sequence of (a) –(e) where T is U.

The term "sequence that has substantial sequence homology" means those nucleic acid sequences which have slight or inconsequential sequence variations from the sequences in (a) or (b), i.e., the sequences function in 25 substantially the same manner. The variations may be attributable to local mutations or structural modifications. Nucleic acid sequences having substantial homology include nucleic acid sequences having at least 65%, more preferably at least 85%, and most preferably 90-95% identity with the nucleic acid sequences as listed in (a) above. The term "sequence that 30 hybridizes" means a nucleic acid sequence that can hybridize to a sequence of (a), (b), (c) or (d) under stringent hybridization conditions. Appropriate "stringent hybridization conditions" which promote DNA hybridization are

-19 -

known to those skilled in the art, or may be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the following may be employed: 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C; 0.2 x SSC at 50°C to 5 65°C; or 2.0 x SSC at 44°C to 50°C. The stringency may be selected based on the conditions used in the wash step. For example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

10 The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

The term "a nucleic acid sequence which is an analog" means a nucleic acid sequence which has been modified as compared to the sequence of (a), (b) or (c) wherein the modification does not alter the utility of the 15 sequence as described herein. The modified sequence or analog may have improved properties over the sequence shown in (a), (b) or (c). One example of a modification to prepare an analog is to replace one of the naturally occurring bases (i.e. adenine, guanine, cytosine or thymidine) of the sequence with a modified base such as such as xanthine, hypoxanthine, 2- 20 aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8 amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl 25 guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Another example of a modification is to include modified phosphorous or oxygen heteroatoms in the phosphate backbone, short chain alkyl or 30 cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages in the nucleic acid molecule listed in (a) to (e) above. For

-20 -

example, the nucleic acid sequences may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates.

A further example of an analog of a nucleic acid molecule of the invention is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogs have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other nucleic acid analogs may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Pat. No. 5,034,506). The analogs may also contain groups such as reporter groups, a group for improving the pharmacokinetic or pharmacodynamic properties of nucleic acid sequence.

Isolated and purified nucleic acid molecules having sequences which differ from the nucleic acid sequence of the invention due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent peptides but differ in sequence from the above mentioned sequences due to degeneracy in the genetic code.

An isolated nucleic acid molecule of the invention which consists of DNA can be isolated by preparing a labeled nucleic acid probe based on all or part of the nucleic acid sequences of the invention and using this labeled nucleic acid probe to screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). For example, a genomic library isolated can be used to isolate a DNA encoding a novel peptide of the invention by screening the library with the labeled probe using standard techniques. Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.

An isolated nucleic acid molecule of the invention which is DNA can also be isolated by selectively amplifying a nucleic acid encoding a novel peptide of the invention using the polymerase chain reaction (PCR) methods

-21 -

and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleic acid sequence of the invention for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., *Biochemistry*, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Invitrogen, Carlsbad, CA, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

An isolated nucleic acid molecule of the invention which is RNA can be isolated by cloning a cDNA encoding a novel peptide of the invention into an appropriate vector which allows for transcription of the cDNA to produce an RNA molecule which encodes a protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g., a T7 promoter) in a vector, cDNA can be transcribed *in vitro* with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

A nucleic acid molecule of the invention may also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071).

Determination of whether a particular nucleic acid molecule encodes a novel peptide of the invention may be accomplished by expressing the cDNA in an appropriate host cell by standard techniques, and testing the activity of the peptide using the methods as described herein. A cDNA having the activity of a novel peptide of the invention so isolated can be sequenced by

-22 -

standard techniques, such as dideoxynucleotide chain termination or Maxam-Gilbert chemical sequencing, to determine the nucleic acid sequence and the predicted amino acid sequence of the encoded peptide.

The initiation codon and untranslated sequences of nucleic acid molecules of the invention may be determined using currently available computer software designed for the purpose, such as PC/Gene (IntelliGenetics Inc., Calif.). Regulatory elements can be identified using conventional techniques. The function of the elements can be confirmed by using these elements to express a reporter gene which is operatively linked to the elements. These constructs may be introduced into cultured cells using standard procedures. In addition to identifying regulatory elements in DNA, such constructs may also be used to identify proteins interacting with the elements, using techniques known in the art.

The sequence of a nucleic acid molecule of the invention may be inverted relative to its normal presentation for transcription to produce an antisense nucleic acid molecule which are more fully described herein. In particular, the nucleic acid sequences contained in the nucleic acid molecules of the invention or a fragment thereof, may be inverted relative to its normal presentation for transcription to produce antisense nucleic acid molecules.

The invention also provides nucleic acids encoding fusion proteins comprising a novel protein of the invention and a selected protein, or a selectable marker protein (see below).

Also provided are portions of the nucleic acid sequence encoding fragments, functional domains or antigenic determinants of the TCAP peptide. The present invention also provides for the use of portions of the sequence as probes and PCR primers for TCAP as well as for determining functional aspects of the sequence.

One of ordinary skill in the art is now enabled to identify and isolate TCAP encoding nucleic acids or cDNAs that are allelic variants of the disclosed sequences, using standard hybridization screening or PCR techniques.

II. Novel Proteins of the Invention

-23 -

The invention further broadly contemplates an isolated TCAP peptide. The term "TCAP peptide" as used herein includes all homologs, analogs, fragments or derivatives of the TCAP peptide.

The term "analog" in reference to peptides includes any peptide
5 having an amino acid residue sequence substantially identical to the human or mouse TCAP sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to mimic TCAP as described herein. Examples of conservative substitutions include the substitution of one non-
10 polar (hydrophobic) residue such as alanine, isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one
15 acidic residue, such as aspartic acid or glutamic acid for another. The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that such polypeptide displays the requisite activity.

20 The term "derivative" reference to peptides refers to a peptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups,
25 chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as derivatives are those peptides
30 which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be

-24 -

substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions or residues relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity
5 is maintained.

In one embodiment, the isolated TCAP peptide consists of 38-41 amino acid residues of the carboxy terminus of a teneurin-like protein with or without an amidation signal at the carboxy terminus. In one embodiment, the amidation signal consists of the amino acid sequence GKR or GRR
10 (preTCAP). In another embodiment, the TCAP peptide comprises sequences substantially identity to the above-noted peptides or comprising an obvious chemical equivalents thereof. It also includes peptides sequence +/- amino acids at the amino and/or carboxy terminus of the above-noted TCAP peptide sequences. In yet another embodiment, the invention includes fusion proteins,
15 comprising the TCAP peptide, labeled TCAP peptides, analogs, homologs and variants thereof.

In one embodiment, the TCAP peptide is a rainbow trout, zebrafish, human, mouse, *G. gallus* or *D. melanogaster* TCAP. In another embodiment, the TCAP peptides have the sequence selected from the group consisting of:
20 SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or that further has an amidation signal sequence (preferably GKR or GRR), at the carboxy terminus of said peptides, such as 15, 16, 23, 24, 31, 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96;

25 In another embodiment, the peptide of the invention is a TCAP-3 and has the following amino acid sequence motif:

QLLSXaa₁Xaa₂KVXaa₃GYDGYVLSXaa₄EQYPELADSANNXaa₅QFL
RQSEI SEQ. ID. NO:135

Where Xaa₁ is G, S, or A; Xaa₂ is G or R; Xaa₃ is L or Q; Xaa₄ and
30 Xaa₅ are independently V or I. In one embodiment, the TCAP-3 is a human or mouse TCAP- 3. In another embodiment, the TCAP- 3 has SEQ. ID. NO: 13, 21, 53 or 85.

-25 -

Within the context of the present invention, a peptide of the invention may include various structural forms of the primary peptide which retain biological activity. For example, a peptide of the invention may be in the form of acidic or basic salts or in neutral form. In addition, individual amino acid
5 residues may be modified by oxidation or reduction.

In addition to the full-length amino acid sequence, the peptide of the present invention may also include truncations, analogs and homologs of the peptide and truncations thereof as described herein. Truncated peptides or fragments may comprise peptides of at least 5, preferably 10 and more
10 preferably 15 amino acid residues of the sequence listed above.

The invention further provides polypeptides comprising at least one functional domain or at least one antigenic determinant of a TCAP peptide.

Analogous of the protein of the invention and/or truncations thereof as described herein, may include, but are not limited to an amino acid sequence
15 containing one or more amino acid substitutions, insertions, deletions and/or mutations. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids of the peptides of the invention with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved
20 substitutions are made the resulting analog should be functionally equivalent. Non-conserved substitutions involve replacing one or more amino acids of the amino acid sequence with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.

One or more amino acid insertions may be introduced into the amino
25 acid sequences of the invention. Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy target sequences so that the peptide is no longer active. This procedure may be used *in vivo* to inhibit the activity of the peptide of the invention.

30 Deletions may consist of the removal of one or more amino acids, or discrete portions from the amino acid sequence of the TCAP peptide. The deleted amino acids may or may not be contiguous.

-26 -

Analogs of a protein of the invention may be prepared by introducing mutations in the nucleotide sequence encoding the peptide. Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Deletion or truncation of a peptide of the invention may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA religated. Exemplary methods of making the alterations set forth above are disclosed by Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989).

The peptides of the invention also include homologs of the amino acid sequence of the TCAP peptide, mutated TCAP peptides and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are comprised of amino acid sequences that hybridize under stringent hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain a peptide of the invention. Homologs of a peptide of the invention will have the same regions which are characteristic of the protein.

A homologous peptide includes a peptide with an amino acid sequence having at least 70%, preferably 80-95% identity with the amino acid sequence of the TCAP peptide.

The invention also contemplates isoforms of the peptides of the invention. An isoform contains the same number and kinds of amino acids as a peptide of the invention, but the isoform has a different molecular structure. The isoforms contemplated by the present invention are those having the same properties as a peptide of the invention as described herein.

-27 -

The proteins of the invention (including e.g., truncations, analogs, etc.) may be prepared using recombinant DNA methods. Accordingly, nucleic acid molecules of the present invention having a sequence that encodes a peptide of the invention may be incorporated according to procedures known in the art
5 into an appropriate expression vector that ensures good expression of the peptide. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression "vectors suitable for
10 transformation of a host cell", means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences, selected on the basis of the host cells to be used for expression, which are operatively linked to the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is linked to regulatory sequences in a manner that allows
15 expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted peptide-sequence. Suitable
20 regulatory sequences may be derived from a variety of sources, including bacterial, fungal, or viral genes (For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequences is dependent on the host cell chosen, and may be
25 readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication,
30 additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It

-28 -

will also be appreciated that the necessary regulatory sequences may be supplied by the native peptide and/or its flanking regions.

The invention further provides a recombinant expression vector comprising a DNA nucleic acid molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression, by transcription of the DNA molecule, of an RNA molecule which is antisense to a nucleotide sequence of the invention. Regulatory sequences operatively linked to the antisense nucleic acid can be chosen which direct the continuous expression of the antisense RNA molecule.

The recombinant expression vectors of the invention may also contain a selectable marker gene that facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

Recombinant expression vectors can be introduced into host cells to produce a transformed host cell. Accordingly, the invention includes a host cell comprising a recombinant expression vector of the invention. The term "transformed host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression

-29 -

vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, 5 for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells 10 can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other such laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the peptides of the invention may be expressed in 15 bacterial cells such as *E. coli*, *Pseudomonas*, *Bacillus subtilis*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991).

As an example, to produce TCAP peptides recombinantly, for example, 20 *E. coli* can be used using the T7 RNA polymerase/promoter system using two plasmids or by labeling of plasmid-encoded proteins, or by expression by infection with M13 Phage mGPI-2. *E. coli* vectors can also be used with Phage lambda regulatory sequences, by fusion protein vectors (e.g. lacZ and trpE), by maltose-binding protein fusions, and by glutathione-S-transferase 25 fusion proteins.

Alternatively, a TCAP peptide can be expressed in insect cells using baculoviral vectors, or in mammalian cells using vaccinia virus. For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters and introduced into cells, such as COS cells to 30 achieve transient or long-term expression. The stable integration of the chimeric gene construct may be maintained in mammalian cells by biochemical selection, such as neomycin and mycophenolic acid.

-30 -

The TCAP DNA sequence can be altered using procedures such as restriction enzyme digestion, fill-in with DNA polymerase, deletion by exonuclease, extension by terminal deoxynucleotide transferase, ligation of synthetic or cloned DNA sequences, site-directed sequence alteration with the
5 use of specific oligonucleotides together with PCR.

The cDNA sequence or portions thereof, or a mini gene consisting of a cDNA with an intron and its own promoter, is introduced into eukaryotic expression vectors by conventional techniques. These vectors permit the transcription of the cDNA in eukaryotic cells by providing regulatory sequences
10 that initiate and enhance the transcription of the cDNA and ensure its proper splicing and polyadenylation. The endogenous TCAP gene promoter can also be used. Different promoters within vectors have different activities which alters the level of expression of the cDNA. In addition, certain promoters can also modulate function such as the glucocorticoid-responsive promoter from
15 the mouse mammary tumor virus.

Some of the vectors listed contain selectable markers or neo bacterial genes that permit isolation of cells by chemical selection. Stable long-term vectors can be maintained in cells as episomal, freely replicating entities by using regulatory elements of viruses. Cell lines can also be produced which
20 have integrated the vector into the genomic DNA. In this manner, the gene product is produced on a continuous basis.

Vectors are introduced into recipient cells by various methods including calcium phosphate, strontium phosphate, electroporation, lipofection, DEAE dextran, microinjection, or by protoplast fusion. Alternatively, the cDNA can
25 be introduced by infection using viral vectors.

TCAP peptides may also be isolated from cells or tissues, including mammalian cells or tissues, in which the peptide is normally expressed.

The protein may be purified by conventional purification methods known to those in the art, such as chromatography methods, high performance liquid
30 chromatography methods or precipitation.

For example, an anti-TCAP antibody (as described below) may be used to isolate a TCAP peptide, which is then purified by standard methods.

-31 -

The peptides of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic
5 Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

III. Uses

The present invention includes all uses of the nucleic acid molecules, TCAP peptides and preTCAP peptides of the invention including, but not limited to, the preparation of antibodies and antisense oligonucleotides, the
10 preparation of experimental systems to study TCAP, the isolation of substances that can bind or modulate TCAP expression and/or activity as well as the use of the TCAP nucleic acid sequences and peptides and modulators thereof in diagnostic and therapeutic applications. Some of the uses are further described below.

15 (a) Antibodies

The isolation of the TCAP peptide enables the preparation of antibodies specific for TCAP. Accordingly, the present invention provides an antibody that binds to a TCAP peptide and/or a protein containing a TCAP peptide, such as preTCAP.

20 Conventional methods can be used to prepare the antibodies. For example, by using a TCAP, polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring
25 immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the protein or peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as
30 antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

-32 -

To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g., the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., Science 246, 1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated. Therefore, the invention also contemplates hybridoma cells secreting monoclonal antibodies with specificity for TCAP.

The term "antibody" as used herein is intended to include fragments thereof which also specifically react with TCAP. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment can be further treated to produce Fab' fragments.

Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes the gene product of TCAP antigen of the invention (See, for example, Morrison et al., Proc. Natl Acad. Sci. U.S.A. 81,6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent

-33 -

Publication 0173494, United Kingdom patent GB 2177096B). It is expected that chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody.

Monoclonal or chimeric antibodies specifically reactive with a peptide of the invention as described herein can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such immunoglobulin molecules may be made by techniques known in the art, (e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80, 7308-7312 (1983); Kozbor et al., Immunology Today, 4, 7279 (1983); Olsson et al., Meth. Enzymol., 92, 3-16 (1982)), and PCT Publication WO92/06193 or EP 0239400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments, reactive against TCAP peptide may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with peptides produced from the nucleic acid molecules encoding TCAP. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., Nature 341, 544-546: (1989); Huse et al., Science 246, 1275-1281 (1989); and McCafferty et al. Nature 348, 552-554 (1990)). Alternatively, a SCID-hu mouse, for example the model developed by Genpharm, can be used to produce antibodies or fragments thereof.

(b) Antisense Oligonucleotides

Isolation of a nucleic acid molecule encoding TCAP enables the production of antisense oligonucleotides that can modulate the expression and/or activity of TCAP. Accordingly, the present invention provides an antisense oligonucleotide that is complimentary to a nucleic acid sequence encoding TCAP.

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complimentary to its target.

-34 -

The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligonucleotides may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells), or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Other antisense oligonucleotides of the invention may contain modified phosphorous, oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates. In an embodiment of the invention there are

-35 -

phosphorothioate bonds links between the four to six 3'-terminus bases. In another embodiment phosphorothioate bonds link all the nucleotides.

The antisense oligonucleotides of the invention may also comprise nucleotide analogs that may be better suited as therapeutic or experimental reagents. An example of an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other oligonucleotides may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Pat. No. 5,034,506). Oligonucleotides may also contain groups such as reporter groups, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an antisense oligonucleotide. Antisense oligonucleotides may also have sugar mimetics.

The antisense nucleic acid molecules may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

-36 -

The antisense oligonucleotides may be introduced into tissues or cells using techniques in the art including vectors (retroviral vectors, adenoviral vectors and DNA virus vectors) or physical techniques such as microinjection. The antisense oligonucleotides may be directly administered *in vivo* or may be
5 used to transfect cells *in vitro* which are then administered *in vivo*. In one embodiment, the antisense oligonucleotide may be delivered to macrophages and/or endothelial cells in a liposome formulation.

(c) Diagnostic Assays

The findings by the present inventors that TCAP is involved in inhibiting
10 neuronal cell proliferation, in inducing an angiogenic response and in inhibiting cell death in cells subject to stress allows development of diagnostic assays, particularly for conditions associated with the aberrant regulation of neuronal growth.

Accordingly, the present invention provides a method of detecting a
15 condition associated with TCAP or preTCAP expression comprising assaying a sample for (a) a nucleic acid molecule encoding a TCAP peptide or a fragment thereof or (b) a TCAP protein or a fragment thereof. The TCAP peptide preferably has a sequence as shown in SEQ.ID.NOS.: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103.
20 In one particular embodiment of the invention the condition is associated with the aberrant regulation of neuronal growth. Neuronal growth may include somatic and process development, mitogenesis or migration. Aberrant regulation of neuronal growth may occur via a disturbance in interneuronal connections and the associated signal molecules. Examples of such
25 conditions include learning deficits, mental retardation, autism, schizophrenia, Alzheimer's Disease, Parkinson's Disease as well as affective disorders such as panic disorder, depression, anorexia nervosa and obsessive-compulsive disorder.

(i) Nucleic acid molecules

30 The nucleic acid molecules encoding TCAP as described herein or fragments thereof, allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences encoding TCAP or fragments

-37 -

thereof in samples, preferably biological samples such as cells, tissues and bodily fluids. The probes can be useful in detecting the presence of a condition associated with TCAP expression or monitoring the progress of such a condition. Accordingly, the present invention provides a method for
5 detecting a nucleic acid molecule encoding a TCAP comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic acid molecule to form a hybridization product, under conditions which permit the formation of the hybridization product, preferably under stringent conditions, and assaying for the hybridization product.

10 Example of probes that may be used in the above method include fragments of the nucleic acid sequences shown in SEQ.ID.NOS.: -18-20, 25-28, 33-36, 41-44, 49-52, 57-60, 65-68, 73-76, 81-84, 89-92, 97-100 or that wherein T can also be U or that encodes a peptide having an amino acid sequence selected from the group consisting of : SEQ. ID. NOS: 13, 14, 21,
15 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or that further has an amidation signal sequence (preferably GKR or GRR), at the carboxy terminus of said peptides, such as 15, 16, 23, 24, 31, 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96. A nucleotide probe may be labelled with a detectable substance such as a radioactive label
20 which provides for an adequate signal and has sufficient half-life such as ^{32}P , ^3H , ^{14}C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compounds, enzymes, antibodies specific for a labelled antigen, and chemiluminescence. An appropriate label may be selected having regard to
25 the rate of hybridization and binding of the probe to the nucleic acid to be detected and the amount of nucleic acid available for hybridization. Labelled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The
30 nucleotide probes may be used to detect genes, preferably in human cells, that hybridize to the nucleic acid molecule of the present invention preferably,

-38 -

nucleic acid molecules which hybridize to the nucleic acid molecule of the invention under stringent hybridization conditions as described herein.

Nucleic acid molecules encoding a TCAP peptide can be selectively amplified in a sample using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleotide sequence shown in Figures 1 – 5 for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using oligonucleotide primers and standard PCR amplification techniques. The amplified nucleic acid can be cloned into an appropriate vector and characterized by DNA sequence analysis. cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., *Biochemistry*, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

Patients may be screened routinely using probes to detect the presence of a TCAP gene by a variety of techniques. Genomic DNA used for the diagnosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for detection of a specific sequence or may be PCR amplified prior to analysis. RNA or cDNA may also be used. To detect a specific DNA sequence hybridization using specific oligonucleotides, direct DNA sequencing, restriction enzyme digest, RNase protection, chemical cleavage, and ligase-mediated detection are all methods which can be utilized. Oligonucleotides specific to mutant sequences can be chemically synthesized and labelled radioactively with isotopes, or non-radioactively using biotin tags, and hybridized to individual DNA samples immobilized on membranes or other solid-supports by dot-blot or transfer from gels after electrophoresis. The presence or absence of these mutant sequences is then visualized using methods such as autoradiography, fluorometry, or colorimetric reaction.

-39 -

Suitable PCR primers can be generated which are useful for example in amplifying portions of the subject sequence containing identified mutations. Other nucleotide sequence amplification techniques may be used, such as ligation-mediated PCR, anchored PCR and enzymatic amplification as would
5 be understood by those skilled in the art.

Sequence alterations may also generate fortuitous restriction enzyme recognition sites that are revealed by the use of appropriate enzyme digestion followed by gel-blot hybridization. DNA fragments carrying the site (normal or mutant) are detected by their increase or reduction in size, or by the increase
10 or decrease of corresponding restriction fragment numbers. Genomic DNA samples may also be amplified by PCR prior to treatment with the appropriate restriction enzyme and the fragments of different sizes are visualized under UV light in the presence of ethidium bromide after gel electrophoresis.

Genetic testing based on DNA sequence differences may be achieved
15 by detection of alteration in electrophoretic mobility of DNA fragments in gels. Small sequence deletions and insertions can be visualized by high-resolution gel electrophoresis. Small deletions may also be detected as changes in the migration pattern of DNA heteroduplexes in non-denaturing gel electrophoresis. Alternatively, a single base substitution mutation may be
20 detected based on differential primer length in PCR. The PCR products of the normal and mutant gene could be differentially detected in acrylamide gels.

Nuclease protection assays (S1 or ligase-mediated) also reveal sequence changes at specific locations. Alternatively, to confirm or detect a polymorphism restriction mapping changes ligated PCR, ASO, REF-SSCP and
25 SSCP may be used. Both REF-SSCP and SSCP are mobility shift assays that are based upon the change in conformation due to mutations.

DNA fragments may also be visualized by methods in which the individual DNA samples are not immobilized on membranes. The probe and target sequences may be in solution or the probe sequence may be
30 immobilized. Autoradiography, radioactive decay, spectrophotometry, and fluorometry may also be used to identify specific individual genotypes.

(ii) Proteins

-40 -

The TCAP protein may be detected in a sample using antibodies that bind to the protein as described in detail above. Accordingly, the present invention provides a method for detecting a TCAP protein comprising contacting the sample with an antibody that binds to TCAP and which is
5 capable of being detected after it becomes bound to the TCAP in the sample.

Antibodies specifically reactive with TCAP, or derivatives thereof, such as enzyme conjugates or labeled derivatives, may be used to detect TCAP in various biological materials, for example they may be used in any known immunoassays which rely on the binding interaction between an antigenic
10 determinant of TCAP, and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination and histochemical tests. Thus, the antibodies may be used to detect and quantify mutated TCAP in a sample in order to determine its role
15 in particular cellular events or pathological states, and to diagnose and treat such pathological states.

In particular, the antibodies of the invention may be used in immuno-histochemical analyses, for example, at the cellular and sub-subcellular level, to detect TCAP, to localize it to particular cells and tissues and to specific
20 subcellular locations, and to quantitate the level of expression.

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect TCAP. Generally, an antibody of the invention may be labelled with a detectable substance and TCAP may be localised in tissue based upon the presence of the detectable
25 substance. Examples of detectable substances include various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, biotin, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein
30 isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include radioactive iodine I-125, I-

-41 -

131 or 3-H. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualized by electron microscopy.

Indirect methods may also be employed in which the primary antigen-
5 antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against TCAP. By way of example, if the antibody having specificity against TCAP is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labelled with a detectable substance as described herein.

10 Where a radioactive label is used as a detectable substance, TCAP may be localized by autoradiography. The results of autoradiography may be quantitated by determining the density of particles in the autoradiographs by various optical methods, or by counting the grains.

(d) Experimental Systems

15 Eukaryotic expression systems are preferred and can be used for many studies of TCAP encoding genes and gene product(s) including the production of large amounts of the peptide for isolation and purification, to use cells expressing the TCAP peptide as a functional assay system for antibodies generated against the peptide or to test effectiveness of pharmacological
20 agents, to study the function of the normal complete peptide, specific portions of the peptide, or of naturally occurring and artificially produced mutant peptides.

Using the techniques mentioned, the expression vectors containing the TCAP peptide cDNA sequence or portions thereof can be introduced into a
25 variety of mammalian cells from other species or into non-mammalian cells.

The recombinant cloning vector, according to this invention, comprises the selected DNA of the DNA sequences of this invention for expression in a suitable host. The DNA is operatively linked in the vector to an expression control sequence in the recombinant DNA molecule so that TCAP peptide
30 protein can be expressed. The expression control sequence may be selected from the group consisting of sequences that control the expression of genes of eukaryotic cells and their viruses and combinations thereof. The expression

-42 -

control sequence may be selected from the group consisting of the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of the fd coat protein, early and late promoters of TCAP, promoters derived from polyoma, adenovirus, 5 retrovirus, baculovirus, simian virus, 3-phosphoglycerate kinase promoter, yeast acid phosphatase promoters, yeast alpha-mating factors and combinations thereof.

Expression of the TCAP peptide in heterologous cell systems may also be used to demonstrate structure-function relationships as well as to provide 10 cell lines for the purposes of drug screening. Inserting a TCAP DNA sequence into a plasmid expression vector to transfect cells is a useful method to test the influence of the peptide on various cellular biochemical parameters including the identification of substrates as well as activators and inhibitors of the gene. Plasmid expression vectors containing either the entire coding sequence for 15 TCAP, or for portions thereof, can be used in *in vitro* mutagenesis experiments that will identify portions of the protein crucial for function. The DNA sequence can be manipulated in studies to understand the expression of the gene and its product. The changes in the sequence may or may not alter the expression pattern in terms of relative quantities, tissue-specificity and functional 20 properties.

The invention also provides methods for examining the function of the TCAP peptide encoded by the nucleic acid molecules of the invention. Cells, tissues, and non-human animals lacking in expression or partially lacking in expression of the peptide may be developed using recombinant molecules of 25 the invention having specific deletion or insertion mutations in the nucleic acid molecule of the invention. A recombinant molecule may be used to inactivate or alter the endogenous gene by homologous recombination, and thereby create a deficient cell, tissue or animal. Such a mutant cell, tissue or animal may be used to define specific cell populations, developmental patterns and *in* 30 *vivo* processes, normally dependent on the protein encoded by the nucleic acid molecule of the invention.

-43 -

Immortalized TCAP responsive cell lines can also be used to identify modulators of TCAP such as noted in Example 13. It can also be used to identify effect of TCAP and TCAP modulators on particular markers. In so far as these markers are associated with the regulation of a medical condition, 5 TCAP and/or the TCAPmodulators may be used in the diagnosis, regulation, and/or treatment of said medical condition.

(e) TCAP Modulators

In addition to antibodies and antisense oligonucleotides described above, other substances that modulate TCAP expression or activity may also 10 be identified.

(i) Substances that Bind/ModulateTCAP

Substances that affect TCAP activity can be identified based on their ability to bind to TCAP.

Substances which can bind with the TCAP of the invention may be 15 identified by reacting the TCAP with a substance which potentially binds to TCAP, and assaying for complexes, for free substance, or for non-complexed TCAP, or for activation of TCAP. In particular, a yeast two hybrid assay system may be used to identify proteins which interact with TCAP (Fields, S. and Song, O., 1989, Nature, 340:245-247). Systems of analysis which also 20 may be used include ELISA.

Accordingly, the invention provides a method of identifying substances which can bind with TCAP, comprising the steps of:

1. reacting TCAP and a test substance, under conditions which allow for formation of a complex between the TCAP and the test substance, and
- 25 2. assaying for complexes of TCAP and the test substance, for free substance or for non complexed TCAP, wherein the presence of complexes indicates that the test substance is capable of binding TCAP.

In another embodiment the invention provides a method of identifying substances that can modulate TCAP activity, such as by binding to TCAP or a 30 TCAP substrate and thus potentially compete (i.e. inhibit TCAP activity), or enhance TCAP/substrate interaction (i.e enhancing TCAP activity), the method comprising:

-44 -

1. reacting TCAP and a TCAP substrate and a test substance, under conditions which allow for formation of a complex between the TCAP and the TCAP substrate, and
2. assaying for complexes of TCAP and the test substance, TCAP and TCAP substrate, TCAP substrate and test substance, for free substance or for non complexed TCAP or TCAP substrate, wherein the presence of complexes with the test substance indicates that the test substance is capable of binding TCAP or TCAP substrate, as the case may be.

10 In another embodiment, a method of identifying modulators of TCAP comprises the use of a cell line that has known reaction to TCAP that can be monitored and monitoring said reaction in the presence of TCAP and a potential modulator.

15 The TCAP peptide used in the assay may have the amino acid sequence shown in SEQ.ID.NOS:, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or may be a fragment, analog, derivative, homolog or mimetic thereof as described herein.

20 Conditions which permit the formation of substance and TCAP complexes may be selected having regard to factors such as the nature and amounts of the substance and the peptide.

25 The substance-peptide complex, free substance or non-complexed peptides may be isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof. To facilitate the assay of the components, antibody against TCAP or the substance, or labelled TCAP, or a labelled substance may be utilized. The antibodies, proteins, or substances may be labelled with a detectable substance as described above.

30 TCAP, or the substance used in the method of the invention may be insolubilized. For example, TCAP or substance may be bound to a suitable carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-

-45 -

exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The carrier may be in the shape of, for example, a tube, test plate, beads, disc, sphere etc.

5 The insolubilized peptide or substance may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

 The peptide or substance may also be expressed on the surface of a cell using the methods described herein.

10 The invention also contemplates assaying for an antagonist or agonist of the action of TCAP.

 It will be understood that the agonists and antagonists that can be assayed using the methods of the invention may act on one or more of the binding sites on the protein or substance including agonist binding sites,
15 competitive antagonist binding sites, non-competitive antagonist binding sites or allosteric sites.

 The invention also makes it possible to screen for antagonists that inhibit the effects of an agonist of TCAP. Thus, the invention may be used to assay for a substance that competes for the same binding site of TCAP.

20 (ii) Peptide Mimetics

 The present invention also includes peptide mimetics of TCAP. "Peptide mimetics" are structures which serve as substitutes for peptides in interactions between molecules (See Morgan et al (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures which
25 may or may not contain amino acids and/or peptide bonds but retain the structural and functional features of a peptide, or enhancer or inhibitor of the invention. Peptide mimetics also include peptoids, oligopeptoids (Simon et al (1972) Proc. Natl. Acad, Sci USA 89:9367); and peptide libraries containing peptides of a designed length representing all possible sequences of amino
30 acids corresponding to a peptide of the invention.

 Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of

-46 -

side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of inhibitor peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

Peptides of the invention may also be used to identify lead compounds for drug development. The structure of the peptides described herein can be readily determined by a number of methods such as NMR and X-ray crystallography. A comparison of the structures of peptides similar in sequence, but differing in the biological activities they elicit in target molecules can provide information about the structure-activity relationship of the target. Information obtained from the examination of structure-activity relationships can be used to design either modified peptides, or other small molecules or lead compounds that can be tested for predicted properties as related to the target molecule. The activity of the lead compounds can be evaluated using assays similar to those described herein.

Information about structure-activity relationships may also be obtained from co-crystallization studies. In these studies, a peptide with a desired activity is crystallized in association with a target molecule, and the X-ray structure of the complex is determined. The structure can then be compared to the structure of the target molecule in its native state, and information from such a comparison may be used to design compounds expected to possess.

(iii) Drug Screening Methods

-47 -

In accordance with one embodiment, the invention enables a method for screening candidate compounds for their ability to increase or decrease the activity and/or expression of TCAP. The method comprises providing an assay system for assaying TCAP activity, assaying the activity in the presence or
5 absence of the candidate or test compound and determining whether the compound has increased or decreased TCAP activity. Such compounds may be useful in treating conditions associated with aberrant regulation of neuronal growth.

Accordingly, the present invention provides a method for identifying a
10 compound that affects TCAP activity or expression comprising:

- (a) incubating a test compound with a TCAP peptide or a nucleic acid encoding a TCAP peptide; and
- (b) determining an amount of TCAP peptide activity or expression and comparing with a control (i.e. in the absence of the test
15 substance), wherein a change in the TCAP activity or expression as compared to the control indicates that the test compound has an effect on TCAP activity or expression.

In accordance with a further embodiment, the invention enables a method for screening candidate compounds for their ability to increase or
20 decrease expression of a TCAP peptide. The method comprises putting a cell with a candidate compound, wherein the cell includes a regulatory region of a gene encoding TCAP operably joined to a reporter gene coding region, and detecting a change in expression of the reporter gene.

Such compounds can be selected from protein compounds, chemicals
25 and various drugs that are added to the culture medium. After a period of incubation in the presence of a selected test compound(s), the expression of mutated TCAP can be examined by quantifying the levels of TCAP mRNA using standard Northern blotting procedure, as described in the examples included herein, to determine any changes in expression as a result of the test
30 compound. Cell lines transfected with constructs expressing TCAP can also be used to test the function of compounds developed to modify the protein expression.

-48 -

(f) Therapeutic Uses

As previously discussed, TCAP of the invention is involved in cAMP, cGMP activity, neuronal growth and neurological development. Accordingly, the present invention provides a method of treating a condition associated with
5 aberrant regulation of cAMP, cGMP, neuronal growth, neuronal communication, or neuronal cell proliferation comprising the administering to a cell or animal in need thereof, an effective amount of agent that modulates TCAP expression and/or activity.

The term "agent that modulates TCAP expression and/or activity"
10 means any substance that can alter the expression and/or activity of TCAP. Examples of agents which may be used to in administration include: a nucleic acid molecule encoding TCAP; the TCAP peptide as well as fragments, analogs, derivatives or homologs thereof; antibodies; antisense nucleic acids; peptide mimetics; and substances isolated using the screening methods
15 described herein that can result in TCAP levels and/or function consistent with a person without the condition.

The term "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired results.

The term "animal" as used herein includes all members of the animal
20 kingdom that respond to TCAP, preferably mammals, including both human and non-human animals, more preferably humans. In another embodiment, animals include domesticated animals, such as cows, horses, pigs, and sheep, In another embodiment, the animals are from the avian family and include chickens.

25 In accordance with another embodiment, the present invention enables gene therapy as a potential therapeutic approach to a condition, in which normal copies of the TCAP gene are introduced into patients to successfully code for normal TCAP peptide in several different affected cell types.

Retroviral vectors can be used for somatic cell gene therapy especially
30 because of their high efficiency of infection and stable integration and expression. The targeted cells however must be able to divide and the expression of the levels of normal protein or peptide should be high. A TCAP

-49 -

encoding gene can be cloned into a retroviral vector and driven from its endogenous promoter or from the retroviral long terminal repeat or from a promoter specific for the target cell type of interest (such as lymphoid cells). Other viral vectors that can be used include adeno-associated virus, vaccinia virus, bovine papilloma virus, or a herpesvirus such as Epstein-Barr virus. Gene transfer could also be achieved using non-viral means requiring infection *in vitro*. This would include calcium phosphate, DEAE dextran, electroporation, cationic or anionic lipid formulations (liposomes) and protoplast fusion. Although these methods are available, many of these are lower efficiency.

Anti-sense based strategies can be employed to inhibit TCAP gene function and as a basis for therapeutic drug design. The principle is based on the hypothesis that sequence specific suppression of gene expression can be achieved by intracellular hybridization between mRNA and a complementary anti-sense species. It is possible to synthesize anti-sense strand nucleotides that bind the sense strand of RNA or DNA with a high degree of specificity. The formation of a hybrid RNA duplex may interfere with the processing/transport/translation and/or stability of a target mRNA.

Hybridization is required for an antisense effect to occur. Antisense effects have been described using a variety of approaches including the use of antisense oligonucleotides, injection of antisense RNA, DNA and transfection of antisense RNA expression vectors.

Therapeutic antisense nucleotides can be made as oligonucleotides or expressed nucleotides. Oligonucleotides are short single strands of DNA which are usually 15 to 20 nucleic acid bases long. Expressed nucleotides are made using expression vectors such as an adenoviral, retroviral or plasmid vector. The vector is administered to the cells in culture, or to a patient, whose cells then make the antisense nucleotide. Expression vectors can be designed to produce antisense RNA, which can vary in length from a few dozen bases to several thousand.

Antisense effects can be induced by control (sense) sequences. The extent of phenotypic changes is highly variable. Phenotypic effects induced by

-50 -

antisense are based on changes in criteria such as biological endpoints, protein levels, protein activation measurement and target mRNA levels.

(g) Methods And Uses Of TCAP For Modulation Of Stress Responses,

5 Related Conditions And Anxiety

The invention also provides a method of detecting an anxiety disorder in an animal by monitoring the effect of TCAP on said animal. If the anxiety response decreases (anxiolytic) as compared to baseline level, then the animal may have a high anxiety related disorder. If the anxiety response of an animal increases in response to administration of TCAP, then the animal may have a low anxiety disorder.

The invention provides a method for normalizing the anxiety state of an animal by administering TCAP to said animal or up-regulating TCAP expression in said animal.

15 The invention also provides a method of inducing a desired anxiety state in an animal by:

(a) determining whether the animal is a low or high anxiety animal; and

(b) (i) administering an effective amount of TCAP or TCAP agonist (including a substance or nucleic acid molecule that up regulates TCAP expression) to increase anxiety in a low anxiety animal and decrease anxiety in a high anxiety animal; or

(ii) administering an inhibitor of TCAP or TCAP antagonist (including a substance or nucleic acid molecule, such as a TCAP antisense nucleic acid molecule, that down regulates TCAP expression) to increase anxiety in a high anxiety animal and decrease anxiety in a low anxiety animal.

The invention also provides a method of detecting a modulator of TCAP activity comprising, administering TCAP to an animal with a known anxiety state (high or low anxiety), administering the potential modulator to said animal and comparing the response to TCAP in the presence and absence of said substance. If the animal's response to TCAP is different than that of baseline (Animal with TCAP alone, and no substance), then said substance is a

-51 -

modulator of TCAP activity. Such compounds may be used to treat animals with undesired stress or anxiety levels.

In one embodiment, TCAP is TCAP-1, or analog, derivative or fragment thereof with similar biological activity.

5 In another embodiment a modulator of TCAP is administered to modulate or regulate the stress response in an animal.

Stress as used herein is any state that is not homeostasis or metabolic balance. Stress is also used to refer to the general state of stressors provoking stress responses (Sapolsky, 1992). Homeostasis refers to the
10 normal stability of the internal environment (Sapolsky, 1992). A Stressor is defined as anything that disrupts physiological balance, be it physical or psychological (Sapolsky, 1992). For example, a stressor in the behavioural experimentals herein (Examples 10 and 11) is defined as a 120 dB tone using the acoustic startle test.

15 Stress Response as used herein is a physiological or behavioural response to stressor(s). For example, in the behavioural experiments (Examples 10 and 11), stress response is the startle response as measured by the acoustic startle testing apparatus (Med Associates, St. Albans, VT) following presentation of a 120 dB tone.

20 Anxiogenic as used herein means a stimulus, internal or external, that increases behavioural measures of anxiety in generally accepted tests. In Examples 10 and 11 herein, the behavioural measure of anxiety is the startle response as measured by the acoustic startle testing apparatus (Med Associates, St. Albans, VT) following the presentation of a 120 dB tone. An
25 anxiogenic response is an increase in the startle response.

Anxiolytic as used herein means a stimulus, internal or external, that decreases behavioural measures of anxiety in generally accepted tests. . In Examples 10 and 11 herein, the behavioural measure of anxiety is the startle response as measured by the acoustic startle testing apparatus (Med
30 Associates, St. Albans, VT) following the presentation of a 120 dB tone. An anxiolytic response is a decrease in the startle response.

-52 -

Anxiety refers to a generalized state of distress that may be prompted by generalized, non-specific cues, and involves physiological arousal, but often without organized functional behaviour (Lang et al., 2000). Animal models of anxiety attempt to represent some aspect of the etiology, symptomatology, or treatment of these disorders (Menard and Treit, 1999). In the present studies, the acoustic startle response was used as a measure of anxiety (Frankland et al., 1996, 1997). This test measures a simple reflex induced by a loud and unexpected auditory stimulus, and can be measured using standardized equipment (Med Associates, St. Albans, Vermont).

High Anxiety as used herein means an animal, e.g., rat, that has a post-vehicle injection startle response that is greater than the baseline response. An average startle response is calculated for the baseline trials and the post-injection (treatment) test periods. The treatment/baseline ratio is then calculated for each animal, e.g., rat. If this ratio is greater than 1, then the animal is classified as high anxiety.

Low Anxiety as used herein means an animal, e.g. rat, that has a post-vehicle injection startle response that is less than the baseline response. The treatment/baseline ratio is calculated for each animal, e.g. rat, as above. If this ratio is less than 1, then the animal, e.g. rat, is classified as low anxiety.

Normal Anxiety as used herein means an animal, such as a rat that has a post-vehicle injection startle response that is the same as the baseline response. The treatment/baseline ratio is calculated for each rat as above. If this ratio is equal to 1, then the animal, e.g. rat, is classified as normal anxiety.

(h) The Role Of TCAP In The Regulation of Cell Proliferation and in the Treatment of Cancer

In one embodiment, the invention provides a method of regulating cell proliferation by administering an effective amount of TCAP to an animal in need thereof. In another embodiment, the TCAP is administered *in vivo* or *in vitro* to decreasing and/or inhibiting cell proliferation. In one embodiment the cell is cancerous. In another embodiment the cell is a neuronal tumour cell.

-53 -

In one embodiment, TCAP or modulators thereof can be used in the treatment of cancer, such as neuroblastomas or other neuronal tumours.

(i) Pharmaceutical Compositions

The above described substances including nucleic acids encoding
5 TCAP, TCAP peptides, antibodies, and antisense oligonucleotides as well as
other agents that modulate TCAP activity or expression may be formulated
into pharmaceutical compositions for administration to subjects in a biologically
compatible form suitable for administration *in vivo*. By "biologically compatible
form suitable for administration *in vivo*" is meant a form of the substance to be
10 administered in which any toxic effects are outweighed by the therapeutic
effects. The substances may be administered to living organisms including
humans, and animals.

Thus in one embodiment, the invention provides the use of TCAP or
modulator there in the preparation of a medicament for the treatment of TCAP
15 -related or TCAP regulated medical conditions. For instance, in the regulation
of cell proliferation (e.g. cancer), stress, anxiety or neuronal communicative
disorders.

Administration of a therapeutically effective amount of pharmaceutical
compositions of the present invention is defined as an amount effective, at
20 dosages and for periods of time necessary to achieve the desired therapeutic
result. For example, a therapeutically effective amount of a substance may
vary according to factors such as the disease state, age, sex, and weight of
the individual, and the ability of the substance to elicit a desired response in
the individual. Dosage regimes may be adjusted to provide the optimum
25 therapeutic response. For example, several divided doses may be
administered daily or the dose may be proportionally reduced as indicated by
the exigencies of the therapeutic situation.

An active substance may be administered in a convenient manner such
as by injection (subcutaneous, intravenous, etc.), oral administration,
30 inhalation, transdermal application, or rectal administration. Depending on the
route of administration, the active substance may be coated in a material to
protect the compound from the action of enzymes, acids and other natural

-54 -

conditions that may inactivate the compound. If the active substance is a nucleic acid encoding, for example, a TCAP peptide it may be delivered using techniques known in the art.

The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985) or Handbook of Pharmaceutical Additives (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995)). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids. In this regard, reference can be made to U.S. Patent No. 5,843,456. As will also be appreciated by those skilled, administration of substances described herein may be by an inactive viral carrier. In one embodiment TCAP can be administered in a vehicle comprising saline and acetic acid.

20 (j) Kits

The reagents suitable for carrying out the methods of the invention may be packaged into convenient kits providing the necessary materials, packaged into suitable containers. Such kits may include all the reagents required to detect a nucleic acid molecule or peptide of the invention or conjugates of a nucleic acid molecule or peptide of the invention and another substance, such as a potential modulator of TCAP, and/or the detection of an indicator of TCAP activity, such as cAMP or cGMP, in a sample by means of the methods described herein, and optionally suitable supports useful in performing the methods of the invention.

30 In one embodiment of the invention, the kit includes primers which are capable of amplifying a nucleic acid molecule of the invention or a predetermined oligonucleotide fragment thereof, all the reagents required to

-55 -

produce the amplified nucleic acid molecule or predetermined fragment thereof in the polymerase chain reaction, and means for assaying the amplified sequences. In one embodiment, the primers can amplify a nucleic acid encoding a TCAP protein, preferably the protein of SEQ.ID.NO.:

5 The kit may also include restriction enzymes to digest the PCR products. In another embodiment of the invention the kit contains a nucleotide probe which hybridizes with a nucleic acid molecule of the invention, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use. In a further embodiment of
10 the invention, the kit includes antibodies of the invention and reagents required for binding of the antibody to a TCAP peptide of the invention in a sample.

Before testing a sample in accordance with the methods described herein, the sample may be concentrated using techniques known in the art,
15 such as centrifugation and filtration. For the hybridization and/or PCR-based methods described herein, nucleic acids may be extracted from cell extracts of the test sample using techniques known in the art.

The following non-limiting examples are illustrative of the present invention:

20

EXAMPLES

Example 1 Identification of Teneurin C-Terminal Associated Peptide (TCAP)

A. Identification of TCAP mRNA

Cloning of mRNA. A rainbow trout hypothalamic cDNA library was
25 constructed as previously described (Barsyte et al., 1999) using a unidirectional vector (Unizap, Stratagene, La Jolla CA). A total of 600,000 clones were screened using a randomly labelled 305-bp hamster urocortin cDNA probe (Robinson et al., 1999)[SEQ. ID. NO 120 -5'-att cac cgccgc tcg
30 gga tct gag cct gca ggc gag cgg cag cga cgg gaa gac ctt ccg ctg tcc atc gac
ctc aca ttc cac ctg cta cgg acc ctg ctg gag atg gcc cgg aca cag agc caa cgc
gag cga gca gag cag aac cga atc ata ctc aac gcg gtc ggc aag tga tcg gcc cgg
tgt ggg acc cca aaa ggc tcg acc ctt tcc cct acc tac ccc ggg gct gaa gtc acg cga

-56 -

ccg aag tcg gct tag tcc cgc ggt gca gcg cct ccc aga gtt acc ctg aac aat ccc gc-3'.] Primary, secondary and tertiary screens all utilized the same probe. The size of the clones, positive after the final screen, were determined by restriction analysis then sequenced using automated Big Dye methods.

5 Five positive clones were isolated from the rainbow trout hypothalamic library. Of these, one represented a partial sequence of a putative rainbow trout Ten-m3 homologue (Figure 1). The clone was 2986 bases long covering the translated portion of 769 bases]. SEQ. ID. NO. 1 shows a 756 base portion [SEQ. ID. NO. 2 thereof and a 3' untranslated region of 734 bases. 10 The stop codon and translated portion were identified by alignment with the mouse (accession number AB025412)[SEQ. ID. NO: 132], human (accession number AK027474)[SEQ. ID. NO: 133] and zebrafish (accession number AB026976)) [SEQ. ID. NO: 134], Ten M3 orthologues. Based on the human gene sequence (Locus Link ID# 10178) using Locus Link on the NICB server, 15 the rainbow trout sequence included the terminal 6 exons of the gene. The final 3' exon encoded a 251 amino acid residue sequence [SEQ. ID. NO. 3] with a 40-41-residue carboxy-terminal sequence [SEQ. ID. NOS. 13 and 14, respectively] suggestive of a bioactive peptide. A putative amidation signal was indicated by the GKR amino acid motif immediately adjacent to the 40-41 20 residue carboxy terminal sequence and TAA stop codon. 40 residues upstream, a PC-7-like cleavage signal was present immediately followed by a glutamine suggesting that the putative free peptide would begin with a pyroglutamic acid. This cleavage site is not necessarily processed in the normal way and can create a 40 or 41 amino acid residue mature peptide 25 (starting at 43 or 44 amino acid residues upstream from the stop codon).

B. Extraction of Free TCAP Peptide

Tissue Collection: Mouse brains (*Mus musculus*; n=10; 1.8g) were collected and stored at -80°C for one month, at which time they were removed and placed immediately into liquid nitrogen. Brain tissue was 30 crushed using a mortar and pestle and powdered in the presence of liquid nitrogen.

-57 -

Activation of C18 packing material: Bondpack® C18 bulk packing material (1g; 125Å; 37-55µm; Waters Corporation, Milford, MA, USA) was activated with 100% methanol (5ml), vortexed and left to stand (5min.). Excess methanol was removed. C18 was then washed in duplicate with PBS
5 (5ml, pH 7.6). An additional PBS aliquot was added (5ml), vortexed and centrifuged (5000rpm; 5min); the supernatant was discarded.

Tissue Extraction: Acetonitrile (90%) and TFA (0.05%) were added to powdered brains in a 5:1 volume to weight ratio, mixed for 1 hr on an aliquot mixer rocker. The mixture was centrifuged (8000rpm X 20 min.); the
10 supernatant was removed and saved. The remaining solids were back-extracted in acetonitrile (90%) and TFA (0.05%) in 40% of the solvent volume used in the initial extraction, vortexed and centrifuged as described previously. The supernatants were pooled and combined with activated C18 packing material, vortexed, mixed (1hr) and centrifuged (8000rpm X 10min).
15 The supernatant was discarded while the pellet was subjected to three successive, independent acetonitrile extractions of 20%, 50% and 90% respectively. Acetonitrile (5ml) was added to the pellet, vortexed, mixed (20min) and centrifuged (6000rpm X 10min.). Resulting supernatant was saved and concentrated to 800µl on a vacuum concentrator (Brinkman
20 Instruments) for HPLC analysis while the pellet was re-extracted in the same manner.

HPLC Purification of free TCAP in brain extracts

A Beckman model 126 HPLC System Gold (Beckman, Palo Alto, CA), attached to a UV detector module 168 and C18 column (3.5 µm particle size;
25 Waters Inc) was used to purify the TCAP peptide extracted from mouse brains (n=10).

A single injection (800ul) was applied to the column through a 1ml injection loop and carried to the column at a flow rate of 1ml/min using a dual solvent system (A: 0.05% trifluoroacetic acid (TFA); B: 80% acetonitrile,
30 0.05% TFA). Following an initial isocratic period of 10min, mobile phase B was increased from 10% to 60% over 75min, held isocratically for 5 min and

-58 -

returned to 10% over 5 min. Fractions were collected (1ml/fraction), aliquoted (500ul) and concentrated to 50ul for analysis using mass spectrometry.

Example 2 Detection of the Cleaved TCAP in Cell and Tissue Extracts

HPLC as described in Example 1 can be used to detect TCAP. Mass Spectroscopy can also be used. Other detection methods can also be combined with HPLC, Mass Spectroscopy or used on their own, such as radio immunoassays, ELISAs, capillary electrophoresis, immunofluorescence confocal microscopy. Mass spectrometric methods identify molecules on the basis of a charged molecule's (ion) mass to charge ratio. A precise determination of the molecules mass is then determined allowing for identification of the molecule. Larger peptides can be sequenced by subsequent fragmentation of the peptide in a collision chamber. This causes preferential breaking of the peptide bonds. The amino acid and peptide fragments are identified by their mass to charge ratio. Radioimmunoassays or enzyme-linked immunosorbant assays (ELISA) utilize an antiserum specific for the molecule of interest. The molecule (TCAP) competes with a tagged structurally similar reference molecules to bind the antibody. The bound and unbound fractions are separated from each other and the quantity of remaining tagged TCAP is measured. This measurement is proportional to the amount of unlabeled TCAP present. Capillary electrophoresis can also be used to identify TCAP using an antibody reaction. In this method, the unbound component is separated from the bound component by migration in an electric field. Immunofluorescence confocal microscopy utilizes a specific antibody bound to TCAP and a secondary antibody that binds to the primary antibody. The secondary antibody is effectively conjugated to an enzyme that catalyzes a fluorescent reaction upon introduction of the appropriate substrate. The amount of fluorescence is proportional to the amount of TCAP and is measured using digital image analysis.

30 Mass Spectrometry Detection of Peptide

-59 -

Samples were dissolved in 5ul of 1:1 (vol/vol) Acetonitrile:water (plus 0.1%(vol/vol) formic acid). Typically, 2-3ul of each sample was loaded on a glass capillary probe tip and analyzed on a Micromass Q-TOF (hybrid quadrupole time of flight) mass spectrometer (Micromass, Manchester, UK).

5 All spectra were acquired under nanospray, positive-ion mode. For MS measurements the quadrupole RF value was set at 0.5. The scanning region (m/z) was between 200-2000 with a scan time of 1 s and a dwell time of 0.1 s. The data was analyzed using MassLynx program (Micromass, Manchester, UK).

10

Example 3 Synthesis and Solubilization of Peptide

Rainbow trout TCAP-3 [SEQ. ID. NO: 13], wherein the terminal isoleucine (I) was amidated [to give SEQ. ID. NO. 15] was synthesized on an automated peptide synthesizer, Model Novayn Crystal (NovaBiochem, UK Ltd. Nottingham, UK) on PEG-PS resin using continuous flow Fmoc chemistry (Calbiochem-Novabiochem Group, San Diego, CA). Eight times excess diisopropyl ethy amine (Sigma Aldrich Canada Ltd) and four times excess Fmoc-amino acid activated with HATU (O-(7-azabenzotriazol-1-,3,3-tetramethyluronium hexfluorophosphate, Applied Biosystems, Foster City, CA) at a 1:1 (mole/mole) ratio were used during the coupling reaction. The reaction time was 1 hour. A solution of 20% piperidine (Sigma-Aldrich Canada Ltd) in N,N-dimethylformide (DMF; Caledon Laboratories Ltd, Canada was used for the deprotection step in the synthesis cycle. The DMF was purified in-house and used fresh each time as a solvent for the synthesis. The cleavage/deprotection of the final peptide was carried out with trifluoroacetic acid (TFA), thioanisole, 1,2 ethandithiol, m-cresole, triisopropylsilane, and bromotrimethyl silane (Sigma-Aldrich Canada Ltd) at a ratio of 40:10:5:1:1:5. Finally, it was desalted on a Sephadex G-10 column using aqueous 0.1% TFA solution and lyophilized. The peptide structure was confirmed by reverse-phase HPLC, amino acid analysis and atmospheric pressure ionization mass spectrometry. The HPLC and Mass spectrometry can be done as described in

30

-60 -

Examples 1 and 2 herein. See above method. The same method was used to synthesize mouse TCAP-1.

The peptide was solubilized using a number of different methods, however, the best results were obtained using alpha cyclodextrin. Acetic acid (1ul) was added to dry TCAP at room temperature, vortexed and left to stand (30min). Alpha-cyclodextrin (company) was then added in a 4:1 volume to dry weight ratio (0.25ug/ul), vortexed, and concentrated to 10% of the original volume on an Eppendorf Vacufuge at 30 °C for 2h and room temperature for the remainder of the process. Distilled, de-ionized water and physiological saline were then added independently in a 1:1 and 3:1, volume to concentrated volume ratio respectively. This solution (0.5ug/ul) was vortexed and centrifuged (11,000 rpm; 3 min). The supernatant was aliquoted and stored at 4 °C. The same method was used to synthesize and solubilize other TCAPS including mouse TCAP-1.

15 **Example 4 Peptide Sequence Relationships and Phylogeny**

The rainbow trout Teneurin 3 exon including the TCAP portion shows a high degree of conservation among its orthologues in zebrafish, mouse, and humans (Figure 2). However the trout sequences also showed high sequence similarity with four mouse Teneurin protein paralogues designated as Teneurin 1 to 4 (Figure 3) and similarly four human paralogues were found in the sequence data base (Figure 4). All possess a high degree of similarity among members of the protein family. The Teneurin protein family represents a type II transmembrane protein where the carboxy terminus is displayed on the extracellular face of the plasma membrane (Figure 6 A and B). The TCAP portion represents only the C-terminal residues of the protein. The TCAP sequence is highly conserved across vertebrate species and even the *Drosophila* version possesses about 60% sequence identity (accession number AF008228) (Figure 7A and B).

Figure 5 illustrates the preTCAP nucleotide coding sequences for human, mouse, zebrafish and rainbow trout plus the stop codon. The coding

-61 -

sequences for TCAP (40 and 41 amino acid residue sequences) can be easily determined from the figure.

A comparison of the conserved motifs within the primary structure of the TCAP and CRF families show a match (Figure 9). Conserved motifs of I/L-
 5 S-X-X (X)- L/V [SEQ. ID. NO: 129] at the amino terminus, L/V-L/I-X-V/aliphatic residue [SEQ. ID. NO: 130] in the middle and the motif N-I/A-H/basic residue-I/L/F-aliphatic residue [SEQ. ID. NO: 131] at the carboxy terminus. A more compelling gage of similarity, however, is shown by the secondary structure predictions (Figure 10). TCAP shows a highly similar polarity profile in
 10 comparison to others in the peptide superfamily. Hydrophobicity, using a Kyte-Doolittle plot shows a general similarity within the middle and carboxy terminal regions, but a more hydrophobic amino terminal region.

Although CRF and urocortin show high sequence similarity for each other and urocortin 2 and 3 show high similarity, the level of identity between
 15 these two paralogous lineages is only about 11%. The level of identity among TCAP members is about 60% (Figure 8). CRF and TCAP belong to a much larger peptide family that also includes the insect diuretic peptides (Figure 11). Key motifs, outlined in Figure 9 show alignment when the insect diuretic peptides are included.

20 **Example 5 PCR Expression of Teneurin mRNA**

The presence of the Teneurin protein in brain extracts and on cell lines were established using PCR. Primers utilized in this experiment were designed from 3'-ends of the published sequences for mouse Ten-M 1, 2, 3, and 4 [SEQ. ID. NOS: 4 – 7]. The TCAP-1 forward primer (25mer: 5'-
 25 ACGTCAGTGTTGATGGGAGGACTA-3') [SEQ. ID. NO: 121] is complementary to nucleotides 7938- 7962 of Teneurin 1. The Teneurin 1 reverse primer (27mer: 5'-CCTCCTGCCTATTTCACTCTGTCTCAT-3') [SEQ. ID. NO: 122] is specific for nucleotides 8262-8288 of Teneurin 1. The primers were predicted to generate a Ten-M1 PCR product of 351 bps. The Teneurin
 30 2 forward primer (25mer: 5'-TCGAGGGCAAGGACACACACTACTT-3') [SEQ. ID. NO: 123] is complementary to nucleotides 7920-7944 of Teneurin 2. The Teneurin 2 reverse primer (26mer: AAGAACTGGATGTTGCTGCTACTGTC-

-62 -

3') [SEQ. ID. NO: 124] is complementary to nucleotides 8354-8379 of Teneurin 2. The primers were predicted to get a Teneurin 2 PCR product of 460 bps. The Teneurin 3 forward primer (25mer: 5'-CAACAACGCCTTCTACCTGGAGAAC) [SEQ. ID. NO: 12]5 is complementary to nucleotides 7681-7705 of Teneurin 3. The Teneurin 3 reverse primer (21mer: 5'-TGTTGTTGGCACTGTCAGCCA-3') [SEQ. ID. NO: 126] is specific for nucleotides 8139-8159. The predicted PCR product for Teneurin 3 primers is 479 bps. The Teneurin 4 forward primer (23mer: 5'-TTTGCCTCCAGTGGTTCCATCTT-3') [SEQ. ID. NO: 127] is complementary to nucleotides 7868-7890 of Teneurin 4. The Teneurin 4 reverse primer (24mer: 5'-TGGATATTGTTGGCGCTGTCTGAC-3') [SEQ. ID. NO: 128] is complementary to nucleotides 8446-8469 of Teneurin 4. The primers were predicted to generate a Teneurin 4 PCR product of 602 bps.

The total RNA of Gn11 cells was isolated using RNeasy Mini Kit (Qiagen). First strand synthesis was performed by using First-Strand Beads (Amersham Pharmacia Biotech). Briefly, 2 μ g of total RNA was mixed with the first strand reaction beads (include buffer, dNTPs, murine reverse transcriptase, RNAGuard, and RNase/DNase-free BSA) and 0.2 μ g random hexamer pd(N)₆ in a volume of 33 μ l. Extension was carried out for 60 minutes at 37°C.

The PCR for Teneurin 1,2,3,and 4 was performed respectively using 1 μ l cDNA with a final reaction volume of 50 μ l containing 0.2mM each dNTP, 5 μ l 10xbuffer, 1.5mM MgCl, 1ul Taq DNA polymerase, 0.2 μ M each Teneurin primer and 0.1 μ M each GAPDH primer (forward and reverse primers; The expected GAPDH DNA \approx 200bps). The initial denaturation was set over an interval of 3 min at 94°C. After 35 cycles of 1 min. at 94°C, 1 min. at 60°C, and 1 min. at 72°C, a 5 min. extension was performed at 72°C. The PCR products were examined by 1.5% agarose gel electrophoresis. The appropriate size DNAs of Teneurin 1, 2 and 4 were extracted from the gel using DNA extraction kit (MBI-Fermentas). The Teneurin 1, 2 and 4 DNAs recovered from the gel were subcloned by using the TOPO TA Cloning kit (Invitrogen Corporation). Briefly, the pCR[®] 2.1-TOPO plasmids with Teneurin 1, 2 or 4

-63 -

DNA were transformed into chemically competent *E. coli* and cultured on LB agar plates and in liquid LB medium successively. The products were purified by using the Perfectprep Plasmid Midi Kit (Eppendorf). Positive results were selected by digesting the plasmids using the restriction endonuclease EcoRI and then by electrophoresis. The positive plasmids were sequenced commercially using T7 sequencing primer (AGTC Corp, Toronto, Canada).

Results

A positive amplification product was obtained from adult mouse cells for Teneurin 1, 2 and 4 using PCR (Figure 12). Similarly, the same products were obtained using mRNA extracted from the immortalized neuronal line, Gn11. A neuronal cell line isolated from the same tumour, NLT, showed expression of only Teneurin 2 and 4. However, a neuroblastoma cell line, Neuro2a appeared to express all four forms of the Teneurin gene family. The Neuro2a is the least differentiated of the cell lines used. A rat fibroblast cell line, TGR1, also showed the presence of paralogues 1, 2 and 4 (data not shown). The identity of the amplification signal was confirmed by sequence analysis. TCAP-1 primers generated a 351 bps sequence and showed 99.43% coincidence with Teneurin 1 DNA. TCAP-2 primers generated a 455 bps sequence and showed 99.56% coincidence with Teneurin 2 DNA. TCAP-4 primers generated a 602 bps sequence and showed 99.83% coincidence with Teneurin 4 DNA. The TCAP 3 primers amplified a 306 bp sequence from mouse neuroblastoma Neuro2a cells. The amplified sequence possesses a 173-bp deletion upstream of the TCAP cleavage signal. This finding indicates that the TCAP-3 primers are specific, but that the Neuro2a cells appear to possess a variant of Teneurin 3.

Example 6 Cell Proliferation Experiments

Several cell lines were utilized initially to establish a model system for which the TCAP could be evaluated. Initially the mouse neuroblastoma cell line, Neuro2a, the human breast cancer cell line MCF-7, mouse GnRH-secreting immortalized neuron lines NLT and Gn11 COS-7 cells, and the rat fibroblast cell line TGR1. Preliminary studies indicated that the cells were

-64 -

responsive to the effects of TCAP Rainbow Trout TCAP-3, SEQ.ID. NO.:13: amidated [SEQ. ID. NO. 15], in that the cells showed a decrease in cell proliferation (data not shown). The studies were performed essentially in accordance with the cell proliferation studies below. Gn11 and TGR1 cells
5 were selected to be used for further studies.

Pharmacological Test of TCAP on fibroblast Cell Lines TGR1 and HO16.4c: 2 plates containing 3×10^4 TGR1 cells/well and 2 plates containing $3 \leftrightarrow 10^4$ HO16.4c cells/well in full-serum medium were prepared for testing. Each 6-wells in the plate was designed as a testing group. 24 hours later,
10 aliquots (20 μ l) of drugs) were added in a 12-hours interval after changing the medium using fresh full-serum DMEM. The cells were observed through a microscope per 4-hours. The numbers of the two cell lines were found significantly lower in TCAP groups at 48-hrs and 72-hrs stages. Cells were counted at 48 hours and 72 hours after being treated. Two plates containing
15 3×10^4 Gn11 cells/well in full-serum medium were prepared for testing. Each 6-wells in the plate was designed as a testing group. 24 hours later, aliquots (20 μ l) of drugs (vehicle:saline+acetic acid; 10^{-6} M TCAP-3) were added in a 12-hours interval after changing the medium using fresh full-serum DMEM. The cells were observed through a microscope per 4-hours. Cells were
20 counted at 48 hours and 72 hours after being treated.

A concentration of 10^{-6} M of TCAP administered at 0, 12 24 and 36 hours decreased the proliferation of a mouse neuronal cell line (Gn11) (Figure 13A – 48 hrs and 13B – 72 hrs), a rat fibroblast cell line(TGR1) by 50-60% at 48 hours (Figure 14)and a HO16.4c cells at 48 hours relative to the vehicle
25 treated cells (Figure 15).

The ability of TCAP to inhibit cell proliferation in the above-noted cell lines, indicates that the peptide would be useful in the regulations of cell proliferation and associated medical conditions such as in the treatment of cancer TCAP could be used to arrest tumour growth and inhibit metastasis.
30 In a preferred embodiment, TCAP could be used in the treatment of neuronal tumors.

-65 -

Example 7 Cyclic Nucleotide Experiments

I. A. cAMP and cGMP assays

Approximately 10^6 Gn11 cells were treated with 20 μ L of 10^{-9} , 10^{-8} , or 10^{-7} or 10^{-6} M TCAP-1 or TCAP-3 and incubated at 37 C for 10 minutes.

5 Medium and peptide was removed and the cells were lysed using 350 μ L of a 0.1 M HCL 0.1 % Triton X-100 solution. Using the same concentrated HCl and Triton X-100 solution and a provided standard concentrate, five standard solutions were made up with concentrations of 200, 50, 12.5, 3.12 and 0.78 pmol/ml. All reactions were done in triplicates. Wells were set up for blanks,

10 non-specific binding, total activity(TA), zero binding, five standards, and 12 samples. Using a 96-well IgG coated plate, 50 μ L of neutralizing reagent were pipetted into each well except the blanks. 150 μ L of the 0.1 M HCL/0.1 % Triton solution was pipetted into the NSB wells and 100 μ L of this solution was pipetted into the zero binding wells. 100 μ L of the standards and 100 μ L

15 of the samples were pipetted into their respective wells. 50 μ L of conjugate were pipetted into each well except the TA and the blank wells. 50 μ L of the cAMP antibody were pipetted into each well except the TA, blank and NSB wells. The plate was allowed to shake overnight. The following morning, the wells were rinsed three times with a 10 times diluted wash buffer solution. 50

20 μ L of conjugate was added to the TA wells and 200 μ L of p-Npp substrate was added to each well. The plate was covered again and incubated at room temp for one hour. At this point, 50 μ L of stop solution was added to all wells and the absorbance was read at 405 nm using a Spectramax spectrophotometer. Three levels of controls were utilized: A blank tube which

25 provides a measure of any reactivity between p-Npp substrate and IgG coated wells.;TA: measure of activity of alkaline phosphotase in conjugate, if any; NSB: measure of binding of conjugate to plate or to antibody; Bo: measure of binding conjugate to antibody (no sample and conjugate competition).

B. Results

30 In the first set of experiments, Gn11 cells were treated with 10^{-6} M of rtTCAP-3 SEQ. ID. NO:13, amidated [SEQ. ID. NO: 15], see above, rat

-66 -

urocortin or the vehicle, as above (Figure 16A). TCAP reduced cAMP accumulation in these cells to 58.9 ± 4.8 % of the vehicle- treated cells ($p < 0.01$). Urocortin induced a non-significant decrease of 89.2 ± 6.3 % of the control cells. In cGMP accumulation experiments, TCAP reduced cGMP accumulation to 38.5 ± 8.8 % of the control cells ($p < 0.01$) whereas urocortin caused a decrease to 50.0 ± 8.5 % of the control cells. (Figure 16B)

II. A. cAMP Assays

Gn11 cells were treated when the confluence reached 70%. The cells were treated with 10^{-9} , 10^{-8} or 10^{-7} M TCAP, urocortin and vehicle, separately, and incubated in incubator at 37 °C. (Details below) Medium was removed and the cells were washed by PBS one time, and then were lysed using 600 uL of 0.1 M HCL solution. After freezing/thawing 3 times, the samples were transferred into microcentrifuge tubes. At the same time, squeezed the cells by 3 ml syringe and 22G needle 20 times. Centrifuge 4000rpm \times 5 min, the supernatant of each sample was aspirated and kept in the -20 °C freezer until the cAMP or cGMP assay was carried on. Using the same concentrated HCl and a provided standard concentrate, five standard solutions were made up with concentrations of 200, 50, 12.5, 3.12 and 0.78 pmol/ml. All reactions were done in duplicates. Wells were set up for blanks, non-specific binding (NSB), total activity (TA), zero binding (B0), five standards, and all samples. Using a 96-well IgG coated plate, 50 uL of neutralizing reagent were pipetted into each well except the blanks and TA. 150 uL of the 0.1 M HCL was pipetted into the NSB wells and 100 uL of this solution was pipetted into the zero binding wells. 100 uL of the standards and 100 uL of the samples were pipetted into their respective wells. 50 uL of conjugate were pipetted into each well except the TA and the blank wells. 50 uL of the cAMP antibody were pipetted into each well except the TA, blank and NSB wells. The plate was allowed to shake overnight (18h) at 200 rpm at 4 °C. The next day, the wells were rinsed three times with a 10 times diluted wash buffer solution. After each well was dried thoroughly, 5 uL of conjugate was added to the TA wells and 200 uL of p-Npp substrate was added to each well. The plate was covered again and incubated at room temp for one hour without shaking. At

-67 -

this point, 50 uL of stop solution was added to all wells and the absorbance was read at 405 nm and 580nm using a Spectramax spectrophotometer. The data of 580 nm were provided the background of each well, which were subtracted from the data of 405 nm.

5 B. Results

10⁻⁸ M TCAP induced a significant increase in cAMP accumulation at 15 minutes after introduction of the peptide and fell to normal limits within 30 minutes of treatment (Figure 17A). Urocortin was used for the purpose of a positive control. Figure 17B illustrates cAMP levels in Gn11 cells in the presence of 10⁻⁴ M 3-isobutyl-1 methyl xanthine (IBMX), a phosphodiesterase inhibitor used to boost cAMP induced by treatment of 10⁻⁸ MTCAP or urocortin. Figure 17C is a bar graph illustrating cAMP accumulation over 30 minutes in Gn11 cells by administration of various concentrations of TCAP or Urocortin in the presence of IBMX. Figure 17D is a bar graph illustrating inhibition of 10⁻⁶ M forskolin- stimulated cAMP by 10⁻⁸ MTCAP or urocortin.

Example 8 Behavioural Studies

A. Brain Stimulation Reward Behaviour Experiments

Rats can be trained to bar press for electrical stimulation of the lateral hypothalamus which activates cholinergic nuclei of the pontine tegmentum and their projections to dopaminergic paths of the forebrain. Once reliable baseline rates of bar pressing have been established for a given current, the consequences of various drugs for the activity of this cholinergic dopaminergic system can be assessed by making injections of substances intracranially and then observing their effects on rates of self stimulating behaviour. TCAP-3 SEQ. ID. NO: 13, amidated, [SEQ. ID. NO. 15] see above, at a concentrations of 1 nM prepared in physiological saline was injected by canulae into the laterodorsal tegmental nucleus through guide cannulae. The rate of bar pressing was compared to the vehicle treated rats.

30 B. Results

-68 -

A robust inhibition of self-reward stimulus occurred when TCAP at 1 nM (4.2 pg/ul) was injected into the caudal midbrain of rats (Figure 18). In both forebrain (lateral ventricle) and midbrain injections the effect was reversible with the rats behaviour returning to normal limits after about 60
5 minutes.

Example 9 Preliminary *in situ* Hybridization Results

The first *in situ* hybridization data indicate that the Teneurin I gene (TCAP-1) is highly expressed in adult rat brain. The regions of greatest
10 expression occur in the lateral septum, bed nucleus of the stria terminalis ventral medial nucleus of the hypothalamus and ventral premammillary nucleus. Lesser expression occurs in the hippocampus and amygdala. This expression pattern is consistent with peptides regulating the stress response (see above) in emotional and mood disorders. These data indicate that TCAP
15 plays a primary role in stress and anxiety regulation rather than one of neurogenesis and neurodegeneration. The Teneurin 4 (TCAP-4) expression also occurs in the adult brain but Teneurin 1 is stronger.

20 **A. Methods**

The methods were performed as previously described (Simmons et al., 1989; Ericsson et al., 1995) using ³⁵S-labelled antisense and sense (control) probes higher high stringency conditions (50% formamide with final washes at 0.2 SSC at 60 C). The ³⁵S-labelled cRNA probes were generated from 350 bp
25 cDNA of exon 33 including the TCAP portion by *in vitro* transcription with the appropriate polymerases (T3 for antisense and T7 for sense).

B. Results

Results are shown in Figure 20. On the left column is the expression of
30 TCAP-1 mRNA using the antisense probe, and on the right column, the sense probe. A-B. central nucleus of the amygdala (CeA); C-D. bed nucleus of the stria terminalis, medial (BSTM); E-F: premammillary ventral nucleus (PMV).

-69 -

Abbreviations: 3V, third ventricle; fx, fornix; ic, internal capsule; LV, lateral ventricle; MeA, medial nucleus of the amygdala; opt, optic tract; st, stria terminalis. Bars = 300 μ m (A-B) and 500 μ m (C-F)

The in situ hybridization data indicate that the TCAP-1 gene is highly expressed in adult rat brain. The expression of the C-terminal teneurin-1 exon including the TCAP-1 region was restricted to hypothalamic and limbic regions (Fig 20 A-F). The regions of greatest expression occur in the lateral septum, bed nucleus of the stria terminalis ventral medial nucleus of the hypothalamus and ventral premammillary nucleus. Lesser expression occurs in the hippocampus and amygdala. This distribution is consistent with TCAP playing a modulatory role with emotionality, anxiety and motivation. The presence of TCAP-1 expression in the ventral premammillary nucleus is of particular interest as there are no known CRF receptors found in this region (Li et al., 2002). There was no evidence that the TCAP containing exon was expressed in regions associated with neurogenesis, such as the olfactory lobes or subependymal layers of the lateral ventricles. Despite the previous recognition of the teneurin proteins, their expression in adult brain has never been examined. However, teneurin 1 and 4 expression has been observed in the diencephalon of developing mouse, chick and zebrafish brain (Rubin et al., 1999; Ben-Zur et al, 2000; Mieda et al., 1999).

These data support the hypothesis that TCAP primary role is one of stress and anxiety regulation.

Example 10 Chronic TCAP study: The Role of TCAP In Modulating The Stress Response

A. Method

1. Wistar Rats were tested in acoustic startle for baseline response (1 hour test consisting of 60 acoustic startle stimuli, 120 dB, 60 sec inter-stimulus interval), and divided into matched groups to receive either TCAP-1 (10 nmol of mouse TCAP-1, amidated [SEQ. ID. NO. 40] in 3 μ l vehicle intra-cerebroventricularly) or Vehicle (e.g. saline and acetic acid).

-70 -

2. Two days later, rats were tested in acoustic startle, 25 stimuli baseline (120 dB, 60 sec inter-stimulus interval), then injected ICV with 10 nmol TCAP-1 or Vehicle, then acute response was measured for 1h (60 stimuli, 120 dB, 60 sec inter-stimulus interval).
- 5 3. 25 days later, rats were given either TCAP-1 (10 nmol in 3 μ l or vehicle (3 μ l once per day for 5 consecutive days ICV.
4. Rats were left alone for 10 days.
5. On the 10th day, rats were tested for acoustic startle response without TCAP-1.
- 10 On the 11th day, rats were re-tested for startle response, again without TCAP-1, for 60 minutes (60 stimuli, 60 sec inter-stimulus interval, 120 dB). Re-tested in startle 13th and 28th days. The vehicle is the mixture of saline and acetic acid into which TCAP-1 was dissolved . When referring to vehicle, this refers to the solution without the addition of TCAP-1.

15

B. Results

Results are shown in Figure 21 for the 0, 10 and 12 days after the 5 consecutive day ICV of Vehicle (21A) or TCAP-1(21B). Startle responses for animals in the chronic study are shown in Figure 22. The average startle response for the two groups (TCAP-1 and Vehicle) on Day 1, before chronic TCAP treatment is shown in Figure 22A. Figure 22B shows the average startle response for TCAP and vehicle groups over the 60 trials in the session on the 10th day after chronic TCAP treatment. Figure 22C shows the mean baseline startle responses for all animals for TCAP and vehicle groups averaged across all 60 trials.

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Example 11 Acute TCAP Study Acoustic Startle Measurements

A. Method

Male Wistar rats (250-275 g), were surgically implanted with cannulae (23 gauge) bilaterally into the basolateral nuclei of the amygdala (AP -2.8, ML +/- 5.0, DV - 7.2 mm, from bregma). One week later, the animals were habituated to the acoustic startle reflex (ASR) chambers (MED Associates,

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-71 -

grid rod cage measuring 7.5" x 3.6" x 4.2"), consisting of 25 trials of 120 dB stimuli presented randomly with an inter-stimulus interval of 55-65 seconds, duration of 30 msec and frequency of 5000Hz. The same stimulus conditions were used for test days, which consisted of a 25 trial baseline, injection with mouse TCAP-1 (with amidation signal)[SEQ. ID. NO. 40] or vehicle (0.25 μ l/side, flow rate 0.5 μ l/min), and testing for a further 60 trials post-drug. Each rat received vehicle treatment on the first test day then TCAP-1 (e.g. mouse TCAP-1) in a random and counter balanced fashion in subsequent test days, spaced 48h apart. On the final test day, all rats again received vehicle treatment. Following histological analysis of cannulae placements, the data of eight rats was retained for statistical analysis.

From the data, rats were divided into high and low anxiety groups depending upon their treatment/baseline ratio for the vehicle. Animals that scored less than one were considered low anxiety, those scoring more than one were considered high anxiety. There were four animals in each anxiety group.

Results are shown in Figures 23 and 24. Figure 23 is a bar graph illustrating the mean treatment/baseline value for both groups for all concentrations of mouse TCAP-1. A repeated measures ANOVA indicated that the level of significant differences between the two anxiety groups was $P=0.0078$. After TCAP-1 treatment the treatment/baseline ratio of low anxiety was similar to the initial high anxiety value and vice versa. A vehicle injection was performed at the end of the study to show that the effect was due to the TCAP-1 and not to the experience of injection. TCAP 1 concentrations were 3, 30, 300 pmoles. A summary of the effect of amygdala-injected TCAP-1 is illustrated in Figure 24. It was shown that the effect by TCAP-1 on startle response is inversely proportional to the baseline startle response. As such TCAP-1 can be used to normalize startle behaviour or stress response.

30 Discussion

Regardless of the mechanism the synthetic TCAP peptide is potent, in vivo at eliciting a behavioural response in rats. Given the strong expression of

-72 -

TCAP in hypothalamic and limbic regions, the synthetic mouse TCAP-1 peptide with amidation signal was micro injected into the basolateral amygdala to determine effects on acoustic startle in rats. Animals possessing a high treatment-to-baseline ratio (>1) showed a significant ($p<0.05$) decrease in startle magnitude, whereas animals with a low treatment-to-baseline ratio (<1) showed a significant ($p,0.05$) and does dependent increase in startle magnitude (Figure 23). These data indicate that TCAP-1 acts to modulate the effect on startle responses depending on baseline reactivity of the particular animal and can normalize the behaviour associated with acoustic startle. Other neuropeptides that have been demonstrated to increase acoustic startle are CRF (Liang et al., 1992), CCK (Frankland et al., 1997) and SP (Krase et al., 1994/1999). The acoustic startle paradigm is a well-known and extensively used paradigm for assessing the anxiogenic or anxiolytic effects of drugs. This is an ideal paradigm for testing a novel compound since the startle reflex does not involve locomotion, learning, memory, or motivated behaviour of any kind, which could possibly confound the interpretation of the results.

The data presented indicate that TCAP represent a new family of neuropeptides associated with the regulation of anxiety by regulating neuronal function in key regions of the forebrain and limbic system. Previous studies have also suggested a role of the teneurin genes with neural regulation. Human Ten-M1 maps to position Xq25 of the X chromosome (Ben-Zur et al., 1999). This is a region associated with X-linked mental retardation syndromes (Minet et al., 1999). The conditions mapped to this site are characterized by severe mental retardation and may include motor sensory neuropathy, deafness and sometimes seizures and impaired vision.

The regulation of TCAP represent a new target to understand the aetiology of neurological dysfunction and psychiatric illness. The example shows that TCAP can be used in the treatment of stress-related disorders and in other neuropathological conditions.

Example 12 Activity of TCAP on immortalized neurons.

-73 -

A. In vitro assays

Gn11 immortalized neurons were cultured as previously reported (Tellam et al., 1998) Direct cAMP measurements were performed with the nonacetylated version of a commercial kit (Assay Designs, Ann Arbor, MI).
5 After starved by serum-free DMEM for 1 hr and replaced with fresh DMEM without serum, cells were treated for 15min with TCAP, urocortin or vehicle \pm CRFR1 antagonist PD171729 in the continued presence of forskolin (1 μ M) and IBMX (100 μ M. Protein assays: Total protein was determined using the BCA protein assay method (Pierce Co). MTT Assays: Gn11 cells were
10 seeded into 96-well plates and cultured in full serum DMEM until the cells were 30% confluent. Vehicle, 1nM, 10nM or 100nM TCAP-1 were added into each group (n=8). (Figure 25A) The MTT assay (Sigma Chemicals) was performed at 0, 6, 12, 24 and 48 hours. Flow Cytometry: DNA content of the Gn11 cells was quantified by staining with propidium iodide and analyzed on
15 a FACSCAN flow cytometer (Beckman Instruments).

B. Results

Mouse TCAP-1 induced a dose-dependent change in cAMP
20 accumulation in mouse immortalized neurons after 15 minutes. A 1 nM dose increased (p<0.05) cAMP levels 45% over the vehicle-treated cells. In contrast, 100 nM TCAP-1 decreased (p<0.05) cAMP accumulation 40% from control cells (Figure 25A). However, co-treatment with the specific CRF type 1 receptor antagonist, PD171729 failed to completely abolish TCAP's effects at
25 cAMP accumulation. In contrast, the same concentration of antagonist induced a complete inhibition (p<0.01) of urocortin-stimulated cAMP accumulation in these cells (Figure 25B). We have previously established that these cells possess a CRF-R1 receptor (Tellam et al., 1998) but not an R2 receptor (data not shown). Concentrations of 1, 10 and 100 nM of TCAP-1
30 induced a significant increase in total protein concentration after 120 minutes in the same cells (Figure 25C). Mouse TCAP-1 treatment of these cells also induced a dose-dependent effect on cell metabolism. Cellular activity as

-74 -

indicated by mitochondrial activity (MTT assay) showed a significant ($p < 0.05$) increase in activity at 1 nM concentration, but a decrease at 100 nM concentrations (Figure 25D). Similarly, 1 nM TCAP reduced ($p < 0.05$) the incidence of G1 phase after 24 hours whereas a 100 nM dose increased ($p < 0.05$) G1 phase as determined by DNA content analysis.

As such α -helical CRF(9-41) antagonist can modulate TCAP stress response modulating activity.

Example 13 Proteomic Profiling and MicroArray Studies

To determine the effect of TCAP and to develop a cell model system for screening TCAP modulators, diagnostic and conditions related to TCAP and methods of medical treatment, TCAP responsive cell lines were subject to proteomic profiling and microarray analysis. This was done using a non-tumorigenic-derived immortalized murine hypothalamic cell line, N38, which has the marker profile shown in **Table 1**. The effect of TCAP on other immortalized cell lines can be preformed by adapting the method noted below.

A. TCAP Responsive Immortalized Hypothalamic Cell Lines

The TCAP responsive immortalized cell lines used were prepared by Denise Belsham, University of Toronto, by preparing a culture of embryonic hypothalamic cells; infecting said culture with a retrovirus encoding a viral oncogene, large T Antigen, operably linked to a promoter and a selectable marker; isolating transfected cells from non-transfected cells to obtain a culture of immortalized hypothalamic cells; subcloning said immortalized cells into sub-cloned populations; and screening said subcloned populations for expression of specific neuronal markers; and selecting and further cloning a specific population. The immortalized cell lines can then be screened for TCAP responsiveness.

TCAP responsiveness was screened by measuring the functional cAMP response of the immortalized subclones to TCAP. The results are shown in Figure 26. N-15-1, #7 (N7), N-18-1, #11 (N22), and N-15-14, #29

-75 -

(N29) were analyzed for the cAMP response to peptide stimulation. The subclones were split into 24 well plates. Cells were starved for 1 h in DMEM without FBS, then medium was replaced with 0.5 ml fresh DMEM (without FBS) with the compounds as indicated. In Figure 26, neurons were exposed to 10^{-7} M (100 nM) TCAP peptide. All peptides were diluted in DMEM containing IBMX (100 μ M). After a 15 min incubation at 37 °C, 1 ml of ice-cold ethanol was added to each well. Cells were scraped from the plate and kept at -20 °C until the amounts of intracellular cAMP were determined in triplicate by RIA (Biotechnologies Inc., Stoughton, MA) according to the manufacturer's instructions.

10 B. Proteomic Profiling Using TCAP 3

NPY17 (N38) immortalized neurons were treated with 100 nM TCAP-3 and subjected to proteomic profiling. In this procedure, the nuclei of cells are isolated and the proteins extracted. This method provides an indication of proteins that are up or down regulated by a given treatment. The proteomic profile indicated that the majority of proteins up-regulated were associated with cell cycle, metabolism and the stress response. A number of cytoskeletal proteins were also upregulated. This observation is of particular importance as many antidepressants have been shown to increase spine density and arborization of neuronal processes. Such events are regulated by cytoskeletal proteins.

20

Proteomic profiling Up regulated at 12 hours

Protein Processing

Parvulin; protein chaperone

Transcriptional Regulation

Npw28 binding protein

Staufen; mRNA targetting

histone acetylmethyl transferase helicase

Cell Growth, Cycle and Proliferation

MIDA1; cell growth regulator

Smad 5; TGFbeta signalling

STE20-like kinase; apoptosis

Kp78, wnt pathway activation

Integrin linked kinase 1, wnt pathway p53 target protein,

tumor suppressor IGFBP, growth regulation

esp1, cell division

sepiapterin reductase

TGFbeta Bp1, growth regulation Rad23, uv repair protein

Extracellular Matrix

protocadherin gammaB5

talin

Cytoskeleton

alpha actinin4

CLP36, actinin4 interaction

Cell Signalling

PKC iota

B. MicroArray Studies

I. Method

5 RNA isolation

Total RNA (TRNA) was isolated from 3 independent treated and untreated N38 hypothalamic cell cultures, pooled (to reduce the noise), utilizing Trizol Reagent (GIBCO/BRL) following the manufacturer's protocol. The quality of total RNA was assessed using an Agilent 2100 Bioanalyzer
10 (version A.02.01S1232, Agilent Technologies). Only RNA with the OD ratio of 1.99-2.0 at 260/280 was used.

Oligonucleotide Arrays (Hybridization, Staining, and Scanning)

Hybridizations were performed on the Mouse MU74Av2 GeneChip Set
15 (Affymetrix, Santa Clara, CA). Samples were prepared for hybridization according to Affymetrix instructions. Briefly, a primer encoding the T7 RNA polymerase promoter linked to oligo-dT₁₇ was used to prime double-stranded cDNA synthesis from each mRNA sample using Superscript II RNase H⁻ reverse transcriptase (Life Technologies, Rockville, MD). Each purified
20 (Qiaquick kit, Qiagen) double-stranded cDNA was in vitro transcribed using T7 RNA polymerase (T7 kit; Enzo), incorporating biotin-UTP and biotin-CTP (Enzo Biochemicals, New York, NY) into the cRNAs, followed by purification using RNEasy (Qiagen) and quantitated by measuring absorption at 260 nm/280 nm. Samples were fragmented and hybridized to the Chip for 16 h at
25 45°C and scanned (GeneArray scanner, Affymetrix). MicroArray Suite Version 5 (MASv5; Affymetrix) was used to scale intensities across the Genechips to 150 fluorescence units, and to determine expression values for each gene on the chip. The expression value for each gene was determined by calculating the average of differences (perfect match intensity minus
30 mismatch intensity) of the probe pairs in use for the gene.

Data Analysis

-77 -

Gene analysis software: Data analysis was performed using two independent softwares, GeneChip and GeneSpring. To identify differentially expressed transcripts, pairwise comparison analyses were carried out with MicroArray Suite Version 5 (MASv5; Affymetrix). This approach, which is based on the Mann-Whitney pairwise comparison test, allows the ranking of results by concordance, as well as the calculation of significance (P value) of each identified change in gene expression. Statistically significant genes ($P < 0.05$) were selected for further analysis. Moreover, statistically significant changes in mean expression values were determined by importing the data from MASv5 into GeneSpring 5 (Silicon Genetics, Redwood City, CA). A stepwise process was followed, first with normalizations. A per-chip followed by a per-gene normalization in order to facilitate direct comparison of biological differences. Next, a second method of filter using Affymetrix data and p value with cut-off of $P < 0.005$ generated 4,841 genes which were used for subsequent analysis utilizing Hierarchical Clustering, k-means, Self Organization Map (SOM) utilizing GeneSpring 5.0.

II. Results

Further, to demonstrate that the cell lines can be used as a model for studying TCAP responsiveness, modulation, and in screening for TCAP modulators, microarray studies were performed on 1 nM TCAP-1 [SEQ ID NO 5 plus amidation signal GRR at C-terminus] treated N38 hypothalamic cells, which do not possess either CRF receptor subtype (Table 4). RNAs isolated from treated and untreated cells were analyzed on oligonucleotide arrays representing 12,884 mouse genes (Affymetrix, <http://www.affymetrix.com>). Standard filtering ($p < 0.005$) and hierarchical clustering algorithm (average lineage method: GeneSpring software – Silicon Genetics) identified significant changes in the expression of 4,841/12,885 genes with 166 genes showing 1.5 fold down-regulation and 35 genes up-regulation in the TCAP-1-treated cells compared to the untreated cells. At 16 hours post-treatment, a significant decrease occurred among several genes, notably, GAS5, SDPR and CD95 that have been associated with growth arrest or apoptotic events

-78 -

(45-47). In contrast, upregulated genes including MK167, MOP3 and GDAP10 have been associated with cell proliferation and cell cycle modulation (48-50). A G-protein coupled receptor-related signal transduction pathway is indicated by the regulation of genes, CREM, AKAP8, AKAP95 and PDE6A. Downstream effectors of RAS such as EFK1 and RGL were also down regulated. Downregulation of the A kinase anchoring protein AKAP95 but upregulation of AKAP8 suggests that TCAP may act, in part, by changing the targeting pattern of PKA (51). The upregulation in inducible nitric oxide (INOS), a intracellular voltage-gated chloride channel (CLCN3) and the serotonin transporter (SLC6A4) may reflect the down stream actions of cAMP-mediated signal cascade and indicates the potential for TCAP to be involved in neuronal signaling systems. A role in interneuron communication by TCAP is also indicated by the modulation of genes associated with the regulation of vesicle trafficking. Thus, the TCAP responsive cell lines can be used to screen for modulators of neuronal function that affect growth, differentiation and communication.

SUMMARY OF EXPERIMENTAL RESULTS

The teneurin c-terminal associated peptide (TCAP) represents the terminal 40 to 41 residues on all four of the known teneurin (Ten M) proteins. On all four of the teneurins, TCAP shows the greatest sequence homology among the entire exon suggesting that it is under the most stringent physiological constraints of the protein. TCAP is a potent inhibitor of neuronal and fibroblast growth possibly by arresting cell cycle. When injected into rat brain it increased the startle reflex and decreased self-administered reward behaviour and was shown to modulate the stress response. These data indicates that TCAP represents a novel neurohormonal system associated with neuronal growth and development.

The finding of a TCAP-like peptide on the carboxy terminus of a type II transmembrane protein is unusual. Assuming that the protein is only expressed on the extracellular face of the cell, then it is likely that the peptide acts in a paracrine manner to regulate the surrounding cells. All Ten M

-79 -

proteins possess a basic residue in positions -1 and -8 upstream from the putative cleavage site from the peptide. Such a basic residue arrangement is recognized by the prohormone convertase 7 (PC7) family of proteases (Saideh and Chretien, 1997), for the processing of peptide prohormones.

5 Assuming this to be the case, then the requisite PC7-like protein would need to be expressed also on the extracellular face of the cell, or perhaps on the extracellular face of an adjacent cell. Alternatively, the protease may be secreted and act in a more mobile fashion. In any case, the release of the cleaved peptide would unlikely to occur in the bolus seen by vesicular release.

10 It is also conceivable that the Ten-M protein is expressed in vesicles of the regulated pathway where intravesicular proteases could cleave the peptide before exocytosis. However, the synthetic peptide shows a strong tendency to aggregate and precipitate at concentrations higher than 2 ug/ul. This is likely due to the high number (15) of leucines, isoleucines, valine, tyrosines and

15 phenylalanine within the peptide. Peptides that have high vesicular concentrations such as the urocortin-like peptide, sauvagine, found in the skin of a neotropical frog, *Phyllomedusa sauvagei*, tend to have a low proportion of hydrophobic residues (Pallai et al., 1983). Thus this physical characteristic of the TCAP peptide supports its preferential release from the cleavage from the

20 extracellular face of the plasma membrane.

The TCAP portion of the Ten-M proteins appears to be the most highly conserved of the terminal exon of the protein. Such high levels of conservation occur when there are many physiological, biochemical constraints acting upon the sequence to inhibit change. Such resistance to

25 change could result from essential interactions with processing or degrading enzymes, receptors, and/or transport proteins. The level of conservation of 90% between the paralogues in vertebrates is high in comparison to the CRF group of peptides to which TCAP appears to be most closely related.

In any case, a number of other bioactive peptides are initially

30 expressed and processed in the same manner as TCAP. Other bioactive peptides such as tumor necrosis factor (TNF) (Utsumi et al., 1995), Apo-2 ligand (Pitti et al., 1996) and fractalkine (Garton et al., 2001) are processed in

-80 -

this manner. These peptides are directed outward at the end of the C-terminus on the extracellular face. Peptides processed and expressed in this manner have the potential for a variety of endocrine or juxtacrine roles. For example they may act as an adhesion molecule for cells displaying the appropriate receptor. Such actions could be particularly important during the migration of neurons in the developing brain, allowing neurons to be directed to a specific target. Alternatively, the peptide may be cleaved via a membrane-bound or extracellular matrix-associated protease to act as a paracrine/autocrine factor to modulate the actions of surrounding cells. Such a mechanism would be important for cells to protect against low oxygen stresses which occur in ischaemia. All three cytokines appear to be processed by a tumor necrosis factor alpha converting enzyme (TACE, ADAM17). This enzyme is also capable of cleaving the cell-surface ectodomain of the amyloid-beta precursor protein (Skovronsky et al. 2001), thus decreasing the generation of amyloid beta suggesting it may have a role in the aetiology of Alzheimer's disease.

The TCAP peptide appears to regulate several physiological events. In a mouse neuronal cell line, Gn11, and a rat fibroblast cell line, TGR1, treatment of TCAP at concentrations of 10^{-9} to 10^{-6} M could inhibit proliferation in a dose-dependent manner where maximal inhibition occurs at about 60%. There was no evidence of apoptosis or necrosis of the cells and morphology did not differ between treated and untreated cells.

This stress-related studies indicate an ability of the TCAP peptide to inhibit the damage done by environmental stresses on cells that would occur during periods of ischaemia or perhaps various neurodegenerative diseases. Given the decrease of proliferation rate seen in unstressed cells and the apparent increase in stressed cells suggests that TCAP may be acting in part to reduce the metabolic activity of the cell. Other related peptides have a similar effect. For example, urocortin can prevent cell death in primary cardiac myocyte cultures by stimulating the p42/p44 mitogen-activated protein (MAP) kinase pathway (Latchman, 2001). Under stressful conditions such as heat shock (Okosi et al., 1998) or ischaemia (Brar et al., 1999), urocortin mRNA is

-81 -

upregulated in cultured cardiac cells, and is also secreted into the medium (Brar et al., 1999), suggesting that it too, is acting in a paracrine fashion to regulate cell metabolism. This effect is much greater by urocortin than CRF. This is of particular interest given that the urocortin paralogues of the CRF family appear to represent evolutionarily older sequences than CRF (Lovejoy and Balment, 1999). Such paracrine actions on cell metabolism may be then one of the initial and critical functions of the ancestor gene that gave rise to both the TCAP and CRF/urocortin/diuretic group of peptides.

The data obtained so far can be used to delineate a tentative model for the mechanism for TCAP (Figure 19). Initially, a stressor, such as changes in pH, temperature, or O₂ levels, or alternatively, a stress-induced ligand triggers an up-regulation of the Ten-M protein. Such stressors likely act through a number of signal transduction pathways including adenylate cyclase and guanylate cyclase. It is conceivable that the stressor also up-regulates the Ten-M cleaving enzyme such as TACE or PC7. The TCAP ligand is then cleaved from its protein and is free to act in an autocrine and paracrine manner. It binds to a G-protein coupled receptor that subsequently interacts with a G-inhibitory protein. This inhibits cAMP and cGMP production to inhibit activation of the cell. In a dividing neuron this would act to inhibit proliferation or migration, and in an mature non-dividing neuron could manifest as a reduction of synaptic output thereby inhibiting the neurological response of an activated nucleus of cells in the brain.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

-82-

Table 1: Cell Lines Screening

MARKER	N-7	N-22	N-29	N-38			
T antigen	+	+	+	+			
NSE	+	+	+	+			
GFAP	-	-	-	-			
NT	-	-	-	-			
ER alpha	+	+	+	+			
ER beta	+	+	+	+			
Tph	+	+	-	w			
Socs-3	+	+	+	+			
AR	-	-	N/A	+			
G2R	+	+	+	-			
CRF	-	-	-	-			
GnRH	+	+	+	w			
POMC	-	+	+	-			
Gal	+	-	w	-			
Lep Receptor	-	+	+	w			
Agrp	+	+	+	+			
Cart	-	-	-	-			
NPY	-	-	+	+			
proGlu	-	w	w	-			
TH	+	-	+	-			
GHRH	-	+	+	+			
Avp	+	+	w	w			
proTRH	-	-	-	-			
Ucn	-	-	-	-			
MCH	+	N/A	+	+			
orexin	-	-	-	-			
DAT	strong	-	w	-			
CRFR1	-	-	-	-			
CRFR2	-	-	-	-			
Aromatase	-	-	-	strong			
GnRH Receptor	-	-	-	-			
Insulin receptor	+	+	+	+			
Oxytocin	+	+	+	+			
New-1	-	-	-	-			
New-2	-	-	-	-			
New-4	-	-	+	-			
GHS-R	N/A	N/A	N/A	-			
Leptin							
som							
NTR	+	w	N/A	-			
mc3R							
mc4R	N/A	N/A	N/A	-			
NPY-Y1							
NPY-Y2							
CRLR	N/A	N/A	N/A	-			
Ghrelin	+	+	N/A	+			
Ghrelin variant	+	-	N/A	-			

The following abbreviations will have their standard scientific abbreviations: T-Ag, Large T-antigen; NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; SNTX, syntaxin; ER, estrogen receptor; AR, androgen receptor; LepR, leptin receptor b; Glp-2R (also G2R), glucagon-like peptide 2 receptor; SOCS-3, suppressor of cytokine signaling 3; NPY, neuropeptide Y; AGRP, agoutirelated peptide; POMC, proopiomelanocortin; CART, cocaine and amphetamine regulated transcript; MCH, melanin-concentrating hormone; Ucn, urocortin; NT, neurotensin; Gal, galanin; Orx, orexin; DAT, dopamine transporter; CRFR, corticotrophin-releasing factor receptor; proGlu, proglucagon; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin-releasing hormone; GnRHR, gonadotropin-releasing hormone receptor; CRF, corticotrophin-releasing factor; TRH, thyroid-releasing hormone; AVP, arginine vasopressin; OXY, oxytocin; Arom, aromatase; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase; TenM-1 (also New-1); TenM-2 (also New-2); TenM-3 (also New-3); and TenM-4 (also New-4), Teneurin 1-4; GHS-R, growth hormone secretagogue receptor; Lep, leptin; SOM, somatostatin; NTR, neurotensin receptor; MC3R, melanocortin receptor-3; MC4R, melanocortin receptor-4; NPY-Y1, NPY receptor Y1; NPY-Y2, NPY receptor Y2; CRLR, calcitonin receptor like receptor; nd, not done; na, not done; w, weak expression.

-83 -

Table 2: Genes Regulated by TCAP-1 at 16 hours

Cluster	Gene	Affimetrix Probe No.	Acc No. GB	Function	Fold change
Growth/ Differentiation	GASS	98530	AI849615	Growth arrest specific transcript	0.46
	SDPR	160373	AI839175	Serum deprivation response protein	0.57
	PPAN	160802	AA674812	Peter Pan homologue	0.62
	CD95	102921	M83649	Fas antigen	0.61
	CRD-BP	102627	AF061569	CRD-binding protein	0.59
	SSG1	160298	AW122012	Steroid sensitive gene 1	0.62
	DIP1/2	97353	AI837497	DAB2 interacting protein	0.68
	GBP3	103202	AW047476	Guanylate binding protein	0.63
	P202	161173	AV229143	202 interferon activatable protein	0.61
	CAII	103441	AI94248	Casein kinase II	0.61
	INI1B	99924	AW121845	Integrase interacting protein 1B	0.48
	MMP1	100484	X66473	Matrix metalloproteinase 1	0.55
	MMP10	94724	Y13185	Matrix metalloproteinase 10	0.59
	PTK7	92325	AI326889	Receptor protein tyrosine kinase	1.53
	P204	98466	M31419	Interferon activatable protein	1.85
	MKI67	161931	AV309347	Cell cycle protein regulator	1.70
	MOP3	102382	AB014494	Circadian rhythm regulator	1.57
	ST7	160591	AI504013	Suppressor of tumorigenicity	1.97
	GDAP10	94192	Y17860	Ganglioside induced diff. protein 10	1.62
	Signalling/ Communication	ERK1	101834	Z14249	Mitogen activated protein kinase
ALK3		92767	D16250	Bone morphogenic protein receptor	0.60
BMP4		93456	L47480	Bone morphogenic protein-4	0.52
IL1R		93914	M20658	Interleukin 1 receptor	0.60
GR		98818	X04435	Glucocorticoid receptor	0.66
BARK1		104270	AA982714	β adrenergic receptor kinase 1	0.61
CAMIII		92631	M19380	Calmodulin III	0.53
PCDH γ		160976	AA222943	protocadherin γ	0.42
AKAP95		95001	AB028920	A kinase anchor protein 95	0.60
TTF-1IP		161019	W41560	TTF-1 interacting peptide	0.50
CREM β 1		100533	M60285	cAMP-responsive element modulator	1.61
AKAP8		161088	AV171460	A kinase anchor protein 8	1.58
PDE6A		100696	X60664	cGMP Phosphodiesterase α	1.68
INOS		104420	U43428	Inducible nitric oxide synthetase	1.50
FNBX		92754	D49920	Ferredoxin-NADP reductase	1.61
SLC6A4		161695	AV230927	Serotonin transporter	1.53
CLCN3		94465	AF029347	Chloride channel protein 3	1.66
Processing		ARF1	95156	AI1853873	ADP ribosylation factor 1
	CLM2-B	93492	AB013469	Cytohesin-2	0.63
	YIPID	99675	AI839766	Rab-mediated membrane transport	1.88
	RAB10	160149	AI841543	Ras oncogene homologue	1.62
	GP25L2	100074	AW046723	gp25L brings cargo forward from ER	1.53
	AP4S1	104561	AI847561	Adaptor related protein complex	1.52

The change in expression levels is indicated relative to the untreated control cell for the same time period of 16 hours. Values >1.5 fold or <0.70 fold were considered significant.

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-94 -

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1/88

SEQUENCE LISTING

- <110> Lovejoy, David
Chewpoy, R.B.
Barsyte, Dalia
Rotzinger, Susan
- <120> Tereuin C-Terminal Associated Peptides (TCAP)
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2/88

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3/88

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20 25 30

4/88

Asn Arg Glu Lys Pro Trp Leu Trp Phe Ala Thr Ala Lys Ser Leu Ile
 35 40 45

Gly Lys Gly Val Met Leu Ala Val Thr Gln Gly Arg Val Val Thr Asn
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Ala Leu Asn Ile Ala Asn Glu Asp Cys Ile Lys Val Ala Ala Val Leu
 65 70 75 80

Asn Asn Ala Phe Tyr Leu Glu Asp Leu His Phe Thr Val Glu Gly Arg
 85 90 95

Asp Thr His Tyr Phe Ile Lys Thr Ser Leu Pro Glu Ser Asp Leu Gly
 100 105 110

Ala Leu Arg Leu Thr Ser Gly Arg Lys Ser Leu Glu Asn Gly Val Asn
 115 120 125

Val Thr Val Ser Gln Ser Thr Thr Val Val Asn Gly Arg Thr Arg Arg
 130 135 140

Phe Ala Asp Val Glu Leu Gln Tyr Gly Ala Leu Ala Leu His Val Arg
 145 150 155 160

Tyr Gly Met Thr Leu Asp Glu Glu Lys Ala Arg Val Leu Glu Gln Ala
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Arg Gln Lys Ala Leu Ser Ser Ala Trp Ser Arg Glu Gln Gln Arg Val
 180 185 190

Arg Glu Gly Glu Glu Gly Val Arg Leu Trp Thr Glu Gly Glu Lys Arg
 195 200 205

Gln Leu Leu Ser Gly Arg Lys Val Leu Gly Tyr Asp Gly Tyr Tyr Val
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Leu Ser Ile Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
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Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 245 250

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5/88

<211> 252

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<223> Mouse Ten M1

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Cys Leu Glu Gly Gly Lys Gln Pro Arg Phe Ala Ala Val Pro Ser Val
35 40 45

Phe Gly Lys Gly Ile Lys Phe Ala Ile Lys Glu Gly Ile Val Thr Ala
50 55 60

Asp Ile Ile Gly Val Ala Asn Glu Asp Ser Arg Arg Leu Ala Ala Ile
65 70 75 80

Leu Asn Asn Ala His Tyr Leu Glu Asn Leu His Phe Thr Ile Glu Gly
85 90 95

Arg Asp Thr His Tyr Phe Ile Lys Leu Gly Ser Leu Glu Glu Asp Leu
100 105 110

Val Leu Ile Gly Asn Thr Gly Gly Arg Arg Ile Leu Glu Asn Gly Val
115 120 125

Asn Val Thr Val Ser Gln Met Thr Ser Val Leu Asn Gly Arg Thr Arg
130 135 140

Arg Phe Ala Asp Ile Gln Leu Gln His Gly Ala Leu Cys Phe Asn Ile
145 150 155 160

Arg Tyr Gly Thr Thr Val Glu Glu Glu Lys Asn His Val Leu Glu Met
165 170 175

Ala Arg Gln Arg Ala Val Ala Gln Ala Trp Thr Gln Glu Gln Arg Arg

6/88

	180		185		190														
Leu	Gln	Glu	Gly	Glu	Glu	Gly	Thr	Arg	Val	Trp	Thr	Glu	Gly	Glu	Lys				
		195					200					205							
Gln	Gln	Leu	Leu	Gly	Thr	Gly	Arg	Val	Gln	Gly	Tyr	Asp	Gly	Tyr	Phe				
	210					215					220								
Val	Leu	Ser	Val	Glu	Gln	Tyr	Leu	Glu	Leu	Ser	Asp	Ser	Ala	Asn	Asn				
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Ile	His	Phe	Met	Arg	Gln	Ser	Glu	Ile	Gly	Arg	Arg								
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			20					25					30						
Arg	Glu	Lys	Ala	Gly	His	Trp	Phe	Ala	Thr	Thr	Thr	Pro	Ile	Ile	Gly				
		35					40					45							
Lys	Gly	Ile	Met	Phe	Ala	Ile	Lys	Glu	Gly	Arg	Val	Thr	Thr	Gly	Val				
	50					55					60								
Ser	Ser	Ile	Ala	Ser	Glu	Asp	Ser	Arg	Lys	Val	Ala	Ser	Val	Leu	Asn				
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Asn	Ala	Tyr	Tyr	Leu	Asp	Lys	Met	His	Tyr	Ser	Ile	Glu	Gly	Lys	Asp				
				85					90					95					

7/88

Thr His Tyr Phe Val Lys Ile Gly Ala Ala Asp Gly Asp Leu Val Thr
 100 105 110

Leu Gly Thr Thr Ile Gly Arg Lys Val Leu Glu Ser Gly Val Asn Val
 115 120 125

Thr Val Ser Gln Pro Thr Leu Leu Val Asn Gly Arg Thr Arg Arg Phe
 130 135 140

Thr Asn Ile Glu Phe Gln Tyr Ser Thr Leu Leu Leu Ser Ile Arg Tyr
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Gly Leu Thr Pro Asp Thr Leu Asp Glu Glu Lys Ala Arg Val Leu Asp
 165 170 175

Gln Ala Gly Gln Arg Ala Leu Gly Thr Ala Trp Ala Lys Glu Gln Gln
 180 185 190

Lys Ala Arg Asp Gly Arg Glu Gly Ser Arg Leu Trp Thr Glu Gly Glu
 195 200 205

Lys Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr
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Tyr Val Leu Pro Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser
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Asn Ile Gln Phe Leu Arg Gln Asn Glu Met Gly Lys Arg
 245 250

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<213> Artificial Sequence

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8/88

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Gly Ala Glu Gln Ser Trp Leu Trp Phe Ala Thr Val Lys Ser Leu Ile
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Gly Lys Gly Val Met Leu Ala Val Ser Gln Gly Arg Val Gln Thr Asn
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Val Leu Asn Ile Ala Asn Glu Asp Cys Ile Lys Val Ala Ala Val Leu
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Asn Asn Ala Phe Tyr Leu Glu Asn Leu His Phe Thr Ile Glu Gly Lys
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Asp Thr His Tyr Phe Ile Lys Thr Thr Thr Pro Glu Ser Asp Leu Gly
 100 105 110

Thr Leu Arg Leu Thr Ser Gly Arg Lys Ala Leu Glu Asn Gly Ile Asn
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Val Thr Val Ser Gln Ser Thr Thr Val Val Asn Gly Arg Thr Arg Arg
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Tyr Gly Met Thr Leu Asp Glu Glu Lys Ala Arg Ile Leu Glu Gln Ala
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Arg Gln Arg Ala Leu Ala Arg Ala Trp Ala Arg Glu Gln Gln Arg Val
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Arg Asp Gly Glu Glu Gly Ala Arg Leu Trp Thr Glu Gly Glu Lys Arg
 195 200 205

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
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9/88

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Cys Gln Gln Ala Pro Glu Thr Lys Lys Phe Ala Ser Ser Gly Ser Ile
 35 40 45

Phe Gly Lys Gly Val Lys Phe Ala Leu Lys Asp Gly Arg Val Thr Thr
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Asp Ile Ile Ser Val Ala Asn Glu Asp Gly Arg Arg Ile Ala Ala Ile
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Leu Asn Asn Ala His Tyr Leu Glu Asn Leu His Phe Thr Ile Asp Gly
 85 90 95

Val Asp Thr His Tyr Phe Val Lys Pro Gly Pro Ser Glu Gly Asp Leu
 100 105 110

Ala Ile Leu Gly Leu Ser Gly Gly Arg Arg Thr Leu Glu Asn Gly Val
 115 120 125

Asn Val Thr Val Ser Gln Ile Asn Thr Met Leu Ile Gln Leu Gln Tyr
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Lys Val Arg Val Leu Glu Leu Ala Arg Gln Arg Ala Val Arg Gln Ala

10/88

165

170

175

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Cys Leu Glu Gly Gly Lys Gln Pro Arg Phe Ala Ala Val Pro Ser Val
 35 40 45

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 65 70 75 80

11/88

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85 90 95

Arg Asp Thr His Tyr Phe Ile Lys Leu Gly Ser Leu Glu Glu Asp Leu
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245 250

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12/88

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 Arg Glu Lys Ala Gly His Trp Phe Ala Thr Thr Thr Pro Ile Ile Gly
 35 40 45
 Lys Gly Ile Met Phe Ala Ile Lys Glu Gly Arg Val Thr Thr Gly Val
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 Ser Ser Ile Ala Ser Glu Asp Ser Arg Lys Val Ala Ser Val Leu Asn
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 Lys Ala Arg Asp Gly Arg Glu Gly Ser Arg Leu Trp Thr Glu Gly Glu
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 Lys Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr
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13/88

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 35 40 45

Gly Lys Gly Val Met Leu Ala Val Ser Gln Gly Arg Val Gln Thr Asn
 50 55 60

Val Leu Asn Ile Ala Asn Glu Asp Cys Ile Lys Val Ala Ala Val Leu
 65 70 75 80

Asn Asn Ala Phe Tyr Leu Glu Asn Leu His Phe Thr Ile Glu Gly Lys
 85 90 95

Asp Thr His Tyr Phe Ile Lys Thr Thr Thr Pro Glu Ser Asp Leu Gly
 100 105 110

Thr Leu Arg Leu Thr Ser Gly Arg Lys Ala Leu Glu Asn Gly Ile Asn
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14/88

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Arg Gln Arg Ala Leu Ala Arg Ala Trp Ala Arg Glu Gln Gln Arg Val
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 195 200 205

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
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 35 40 45

Phe Gly Lys Gly Val Lys Phe Ala Leu Lys Asp Gly Arg Val Thr Thr
 50 55 60

15/88

Asp Ile Ile Ser Val Ala Asn Glu Asp Gly Arg Arg Val Ala Ala Ile
65 70 75 80

Leu Asn His Ala His Tyr Leu Glu Asn Leu His Phe Thr Ile Asp Gly
85 90 95

Val Asp Thr His Tyr Phe Val Lys Pro Gly Pro Ser Glu Gly Asp Leu
100 105 110

Ala Ile Leu Gly Leu Ser Gly Gly Arg Arg Thr Leu Glu Asn Gly Val
115 120 125

Asn Val Thr Val Ser Gln Ile Asn Thr Val Leu Ser Gly Arg Thr Arg
130 135 140

Arg Tyr Thr Asp Ile Gln Leu Gln Tyr Gly Ala Leu Cys Leu Asn Thr
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Arg Tyr Gly Thr Thr Leu Asp Glu Glu Lys Ala Arg Val Leu Glu Leu
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Ala Arg Gln Arg Ala Val Arg Gln Ala Trp Ala Arg Glu Gln Gln Arg
180 185 190

Leu Arg Glu Gly Glu Glu Gly Leu Arg Ala Trp Thr Glu Gly Glu Lys
195 200 205

Gln Gln Val Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe
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245 250

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16/88

<223> Zebrafish Ten M3

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Ser Arg Glu Lys Pro Trp Leu Trp Phe Ala Thr Val Lys Ser Leu Ile
 35 40 45

Gly Lys Gly Val Met Leu Ala Ile Thr Ser Lys Gly Gln Val Ala Thr
 50 55 60

Asn Ala Leu Asn Ile Ala Asn Glu Asp Cys Ile Lys Val Val Thr Val
 65 70 75 80

Leu Asn Asn Ala Phe Tyr Leu Glu Asp Leu His Phe Thr Val Glu Gly
 85 90 95

Arg Asp Thr His Tyr Phe Ile Lys Thr Ser Leu Pro Glu Ser Asp Leu
 100 105 110

Gly Ala Leu Arg Leu Thr Ser Gly Arg Lys Ser Leu Glu Asn Gly Val
 115 120 125

Asn Val Thr Val Ser Gln Ser Thr Thr Val Val Asn Gly Arg Thr Arg
 130 135 140

Arg Phe Ala Asp Val Glu Leu Gln Tyr Gly Ala Leu Ala Leu His Val
 145 150 155 160

Arg Tyr Gly Met Thr Leu Asp Glu Glu Lys Ala Arg Val Leu Glu Gln
 165 170 175

Ala Arg Gln Arg Ala Leu Ser Ser Ala Trp Ala Arg Glu Gln Gln Arg
 180 185 190

Val Arg Asp Gly Glu Glu Gly Val Arg Leu Trp Thr Glu Gly Glu Lys
 195 200 205

Arg Gln Leu Leu Ser Ser Gly Lys Val Leu Gly Tyr Asp Gly Tyr Tyr
 210 215 220

17/88

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 225 230 235 240

Val Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 245 250

<210> 13

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Rainbow Trout TCAP3 (40a.a.)

<400> 13

Gln Leu Leu Ser Gly Arg Lys Val Leu Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Ile Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

<210> 14

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Rainbow Trout TCAP 3 (41a.a.)

<400> 14

Arg Gln Leu Leu Ser Gly Arg Lys Val Leu Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Ile Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn

18/88

20

25

30

Ile Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

<210> 15

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Rainbow Trout preTCAP3 (43 a.a.)

<400> 15

Gln Leu Leu Ser Gly Arg Lys Val Leu Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Ile Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 35 40

<210> 16

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Rainbow Trout preTCAP3 (44 a.a.)

<400> 16

Arg Gln Leu Leu Ser Gly Arg Lys Val Leu Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Ile Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

19/88

Ile Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 35 40

<210> 17

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Rainbow Trout TCAP3 (120 n.a.)

<400> 17
 cagctgctga gcgggaggaa ggttctgggc tacgacgggt actacgtcct ctccatagag 60
 cagtaccccg agctagcaga ctccgctaac aacatccagt tcctcaggca gagcgaaata 120

<210> 18

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Rainbow Trout TCAP3 (123 n.a.)

<400> 18
 aggcagctgc tgagcgggag gaaggttctg ggctacgacg ggtactacgt cctctccata 60
 gagcagtacc ccgagctagc agactccgct aacaacatcc agttcctcag gcagagcgaa 120
 ata 123

<210> 19

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Rainbow Trout preTCAP3 (129 n.a.)

20/88

<400> 19
 cagctgctga gcgaggaggaa ggttctgggc tacgacgggt actacgtcct ctccatagag 60
 cagtaccccg agctagcaga ctccgctaac aacatccagt tcctcaggca gagcgaaata 120
 gggaagagg 129

<210> 20

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Rainbow Trout preTCAP3 (132 n.a.)

<400> 20
 aggcagctgc tgagcgggag gaaggttctg ggctacgacg ggtactacgt cctctccata 60
 gagcagtacc ccgagctagc agactccgct aacaacatcc agttcctcag gcagagcgaa 120
 atagggaaga gg 132

<210> 21

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP3 (40 a.a.)

<400> 21

Gln Leu Leu Ser Ser Gly Lys Val Leu Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Val
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

21/88

<210> 22

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP3 (41 a.a.)

<400> 22

Arg	Gln	Leu	Leu	Ser	Ser	Gly	Lys	Val	Leu	Gly	Tyr	Asp	Gly	Tyr	Tyr
1				5					10					15	

Val	Leu	Ser	Val	Glu	Gln	Tyr	Pro	Glu	Leu	Ala	Asp	Ser	Ala	Asn	Asn
			20					25					30		

Val	Gln	Phe	Leu	Arg	Gln	Ser	Glu	Ile
		35					40	

<210> 23

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish preTCAP3 (43 a.a.)

<400> 23

Gln	Leu	Leu	Ser	Ser	Gly	Lys	Val	Leu	Gly	Tyr	Asp	Gly	Tyr	Tyr	Val
1				5					10					15	

Leu	Ser	Val	Glu	Gln	Tyr	Pro	Glu	Leu	Ala	Asp	Ser	Ala	Asn	Asn	Val
			20					25					30		

Gln	Phe	Leu	Arg	Gln	Ser	Glu	Ile	Gly	Lys	Arg
		35					40			

<210> 24

<211> 44

22/88

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish preTCAP3 (44 a.a.)

<400> 24

Arg Gln Leu Leu Ser Ser Gly Lys Val Leu Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

Val Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 35 40

<210> 25

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP3 (120 n.a.)

<400> 25

cagttgctca gctctgggaa ggtgctgggt tacgatgggt actatgtact atcagtgagg 60

caataccctg aactggccga cagtgccaac aatgtccagt tcttgaggca gaggtagata 120

<210> 26

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP3 (123 n.a.)

23/88

<400> 26
 aggcagttgc tcagctctgg gaaggtgctg ggttacgatg gttactatgt actatcagtg 60
 gagcaatacc ctgaactggc cgacagtgcc aacaatgtcc agttcttgag gcagagtgag 120
 ata 123

<210> 27

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP3 (129 n.a.)

<400> 27
 cagttgctca gctctgggaa ggtgctgggt tacgatgggt actatgtact atcagtgagg 60
 caataccctg aactggccga cagtgccaac aatgtccagt tcttgaggca gagtgagata 120
 gggaagagg 129

<210> 28

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish preTCAP3 (132 n.a.)

<400> 28
 aggcagttgc tcagctctgg gaaggtgctg ggttacgatg gttactatgt actatcagtg 60
 gagcaatacc ctgaactggc cgacagtgcc aacaatgtcc agttcttgag gcagagtgag 120
 ataggaaga gg 132

<210> 29

<211> 40

<212> PRT

<213> Artificial Sequence

24/88

<220>

<223> Zebrafish TCAP4 (40 a.a.)

<400> 29

Gln Leu Leu Ser Ser Gly Arg Val Gln Gly Tyr Glu Gly Phe Tyr Ile
 1 5 10 15

Val Ser Val Asp Gln Phe Pro Glu Leu Thr Asp Asn Ile Asn Asn Val
 20 25 30

His Phe Trp Arg Gln Thr Glu Met
 35 40

<210> 30

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP4 (41 a.a.)

<400> 30

Gln Gln Leu Leu Ser Ser Gly Arg Val Gln Gly Tyr Glu Gly Phe Tyr
 1 5 10 15

Ile Val Ser Val Asp Gln Phe Pro Glu Leu Thr Asp Asn Ile Asn Asn
 20 25 30

Val His Phe Trp Arg Gln Thr Glu Met
 35 40

<210> 31

<211> 43

<212> PRT

<213> Artificial Sequence

25/88

<220>

<223> Zebrafish preTCAP4 (43 a.a.)

<400> 31

Gln Leu Leu Ser Ser Gly Arg Val Gln Gly Tyr Glu Gly Phe Tyr Ile
 1 5 10 15

Val Ser Val Asp Gln Phe Pro Glu Leu Thr Asp Asn Ile Asn Asn Val
 20 25 30

His Phe Trp Arg Gln Thr Glu Met Gly Arg Arg
 35 40

<210> 32

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish preTCAP4 (44 a.a.)

<400> 32

Gln Gln Leu Leu Ser Ser Gly Arg Val Gln Gly Tyr Glu Gly Phe Tyr
 1 5 10 15

Ile Val Ser Val Asp Gln Phe Pro Glu Leu Thr Asp Asn Ile Asn Asn
 20 25 30

Val His Phe Trp Arg Gln Thr Glu Met Gly Arg Arg
 35 40

<210> 33

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP4 (120 n.a.)

26/88

<400> 33
 cagctcctaa gctctggacg tgtacagggc tacgaaggct tctacatagt atcagtcgac 60
 cagttcccag agttgactga caacataaat aacgtccatt tctggcgaca gactgagatg 120

<210> 34

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP4 (123 n.a.)

<400> 34
 cagcagctcc taagctctgg acgtgtacag ggctacgaag gcttctacat agtatcagtc 60
 gaccagttcc cagagttgac tgacaacata aataacgtcc atttctggcg acagactgag 120
 atg 123

<210> 35

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Zebrafish preTCAP4 (129 n.a.)

<400> 35
 cagctcctaa gctctggacg tgtacagggc tacgaaggct tctacatagt atcagtcgac 60
 cagttcccag agttgactga caacataaat aacgtccatt tctggcgaca gactgagatg 120
 ggacgcagg 129

<210> 36

<211> 132

<212> DNA

<213> Artificial Sequence

27/88

<220>

<223> Zebrafish preTCAP4 (132 n.a.)

<400> 36

cagcagctcc taagctctgg acgtgtacag ggctacgaag gcttctacat agtatcagtc 60

gaccagttcc cagagttgac tgacaacata aataacgtcc atttctggcg acagactgag 120

atgggacgca gg 132

<210> 37

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP1 (40 a.a.)

<400> 37

Gln Leu Leu Gly Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe Val
1 5 10 15Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn Ile
20 25 30His Phe Met Arg Gln Ser Glu Ile
35 40

<210> 38

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP1 (41 a.a.)

<400> 38

Gln Gln Leu Leu Gly Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe

29/88

Val Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Ile Gly Arg Arg
 35 40

<210> 41

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP1 (120 n.a.)

<400> 41
 cagcttttgg gcaccgggag ggtgcagggg tatgatgggt attttgtctt gtctgttgag 60
 cagtatttag aactttcaga cagtgccaac aatattcact tcatgagaca gagtgaaata 120

<210> 42

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP1 (123 n.a.)

<400> 42
 cagcagcttt tgggcaccgg gaggggtgcag gggatatgatg ggtattttgt cttgtctggt 60
 gagcagtatt tagaactttc agacagtgcc aacaatattc acttcatgag acagagtgaa 120
 ata 123

<210> 43

<211> 129

<212> DNA

<213> Artificial Sequence

30/88

<220>

<223> Mouse preTCAP1 (129 n.a.)

<400> 43
cagcttttgg gcaccgggag ggtgcagggg tatgatgggt attttgtctt gtctgttgag 60
cagtatttag aactttcaga cagtgccaac aatattcact tcatgagaca gagtgaaata 120
ggcaggagg 129

<210> 44

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP1 (132 n.a.)

<400> 44
cagcagcttt tgggcaccgg gaggggtgcag gggatatgatg ggtattttgt cttgtctgtt 60
gagcagtatt tagaactttc agacagtgcc aacaatattc acttcatgag acagagtgaa 120
ataggcagga gg 132

<210> 45

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP2 (40 a.a.)

<400> 45

Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr Tyr Val
1 5 10 15

Leu Pro Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser Asn Ile
20 25 30

32/88

<210> 48

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse preTCAP2 (44 a.a.)

<400> 48

Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr Tyr
 1 5 10 15

Val Leu Pro Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Asn Glu Met Gly Lys Arg
 35 40

<210> 49

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP2 (120 n.a.)

<400> 49
 caactcctga gcacgggacg ggtacaaggt tatgagggct attacgtact tccggtggaa 60
 cagtaccggg agctggcaga cagtagcagc aacatccagt tcttaagaca gaatgagagg 120

<210> 50

<211> 123

<212> DNA

<213> Artificial Sequence

33/88

<220>

<223> Mouse TCAP 2 (123 n.a.)

<400> 50

cagcaactcc tgagcacggg acgggtacaa ggttatgagg gctattacgt acttccgggtg 60
 gaacagtacc cggagctggc agacagtagc agcaacatcc agttcttaag acagaatgag 120
 atg 123

<210> 51

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP2 (129 n.a.)

<400> 51

caactcctga gcacgggacg ggtacaaggt tatgagggct attacgtact tccgggtggaa 60
 cagtaccggg agctggcaga cagtagcagc aacatccagt tcttaagaca gaatgagatg 120
 ggaaagagg 129

<210> 52

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP2 (132 n.a.)

<400> 52

cagcaactcc tgagcacggg acgggtacaa ggttatgagg gctattacgt acttccgggtg 60
 gaacagtacc cggagctggc agacagtagc agcaacatcc agttcttaag acagaatgag 120
 atgggaaaga gg 132

<210> 53

<211> 40

34/88

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP3 (40 a.a.)

<400> 53

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

<210> 54

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP3 (41 a..a)

<400> 54

Arg Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

<210> 55

<211> 43

<212> PRT

35/88

<213> Artificial Sequence

<220>

<223> Mouse preTCAP3 (43 a.a.)

<400> 55

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 35 40

<210> 56

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse preTCAP3 (44 a.a.)

<400> 56

Arg Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg
 35 40

<210> 57

<211> 120

<212> DNA

<213> Artificial Sequence

36/88

<220>

<223> Mouse TCAP3 (120 n.a.)

<400> 57
 cagctgctga gcgctggcaa ggtgcagggc tacgatgggt actacgtact gtcggtggag 60
 cagtacccccg agctggctga cagtgccaac aacatccagt tcttgcgaca aagtgagatc 120

<210> 58

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP3 (123 n.a.)

<400> 58
 cggcagctgc tgagcgctgg caaggtgcag ggctacgatg ggtactacgt actgtcggtg 60
 gagcagtacc ccgagctggc tgacagtgcc aacaacatcc agttcttgcg acaaagtgag 120
 atc 123

<210> 59

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP3 (129 n.a.)

<400> 59
 cagctgctga gcgctggcaa ggtgcagggc tacgatgggt actacgtact gtcggtggag 60
 cagtacccccg agctggctga cagtgccaac aacatccagt tcttgcgaca aagtgagatc 120
 ggcaagagg 129

<210> 60

<211> 132

37/88

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP3 (132 n.a.)

<400> 60
 cggcagctgc tgagcgctgg caaggtgcag ggctacgatg ggtactacgt actgtcggtg 60
 gagcagtacc ccgagctggc tgacagtgcc aacaacatcc agttcttgcg acaaagtgag 120
 atcggcaaga gg 132

<210> 61

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP4 (40 a.a.)

<400> 61

Gln Val Leu Asn Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe Val
 1 5 10 15
 Thr Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30
 His Phe Met Arg Gln Ser Glu Met
 35 40

<210> 62

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse TCAP4 (41 a.a.)

38/88

<400> 62

Gln Gln Val Leu Asn Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe
 1 5 10 15

Val Thr Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Met
 35 40

<210> 63

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse preTCAP4 (43 a.a.)

<400> 63

Gln Val Leu Asn Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe Val
 1 5 10 15

Thr Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30

His Phe Met Arg Gln Ser Glu Met Gly Arg Arg
 35 40

<210> 64

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse preTCAP4 (44 a.a.)

<400> 64

39/88

Gln Gln Val Leu Asn Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe
 1 5 10 15

Val Thr Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Met Gly Arg Arg
 35 40

<210> 65

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP4 (120 n.a.)

<400> 65
 caggtgctga acacggggcg ggtgcaaggc tacgacggct tctttgtgac ctcggtcgag 60
 cagtaccag aactgtcaga cagcgccaac aatatccact tcatgagaca gagcgagatg 120

<210> 66

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse TCAP4 (123 n.a.)

<400> 66
 cagcaggtgc tgaacacggg gcgggtgcaa ggctacgacg gcttctttgt gacctcggtc 60
 gagcagtacc cagaactgtc agacagcgcc aacaatatcc acttcatgag acagagcgag 120
 atg 123

<210> 67

<211> 129

<212> DNA

40/88

<213> Artificial Sequence

<220>

<223> Mouse preTCAP4 (129 n.a.)

<400> 67
 caggtgctga acacggggcg ggtgcaaggc tacgacggct tctttgtgac ctcggtcgag 60
 cagtaccag aactgtcaga cagcgccaac aatatccact tcatgagaca gagcgagatg 120
 ggccgaagg 129

<210> 68

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse preTCAP4 (132 n.a.)

<400> 68
 cagcaggtgc tgaacacggg gcgggtgcaa ggctacgacg gcttctttgt gacctcggtc 60
 gagcagtacc cagaactgtc agacagcgcc aacaatatcc acttcatgag acagagcgag 120
 atgggccgaa gg 132

<210> 69

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP1 (40 a.a.)

<400> 69

Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe Val
 1 5 10 15

41/88

Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30

His Phe Met Arg Gln Ser Glu Ile
 35 40

<210> 70

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP1 (41 a.a.)

<400> 70

Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Ile
 35 40

<210> 71

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP1 (43 a.a.)

<400> 71

Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe Val
 1 5 10 15

Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30

42/88

His Phe Met Arg Gln Ser Glu Ile Gly Arg Arg
 35 40

<210> 72

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP1 (44 a.a.)

<400> 72

Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Ile Gly Arg Arg
 35 40

<210> 73

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP1 (120 n.a.)

<400> 73
 cagcttttga gcactgggcg ggtacaaggt tacgatgggt attttgtttt gtctgttgag 60

cagtatttag aactttctga cagtgccaat aatattcact ttatgagaca gagcgaaata 120

<210> 74

<211> 123

<212> DNA

43/88

<213> Artificial Sequence

<220>

<223> Human TCAP1 (123 n.a.)

<400> 74
cagcagcttt tgagcactgg gcgggtacaa ggttacgatg ggtatdddgt tttgtctggt 60
gagcagtatt tagaactttc tgacagtgcc aataatattc actttatgag acagagcgaa 120
ata 123

<210> 75

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP1 (129 n.a.)

<400> 75
cagctdddga gcactgggcg ggtacaaggt tacgatgggt atdddgtddd gtctgdtgag 60
cagtattdag aactdddctga cagtgccaat aatattcact ttatgagaca gagcgaaata 120
ggcaggagg 129

<210> 76

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP1 (132 n.a.)

<400> 76
cagcagcttt tgagcactgg gcgggtacaa ggttacgatg ggtatdddgt tttgtctggt 60
gagcagtatt tagaactttc tgacagtgcc aataatattc actttatgag acagagcgaa 120
ataggcagga gg 132

44/88

<210> 77

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP2 (40 a.a.)

<400> 77

Gln	Leu	Leu	Ser	Thr	Gly	Arg	Val	Gln	Gly	Tyr	Glu	Gly	Tyr	Tyr	Val
1				5					10					15	

Leu	Pro	Val	Glu	Gln	Tyr	Pro	Glu	Leu	Ala	Asp	Ser	Ser	Ser	Asn	Ile
			20					25					30		

Gln	Phe	Leu	Arg	Gln	Asn	Glu	Met
		35				40	

<210> 78

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP2 (41 a.a.)

<400> 78

Gln	Gln	Leu	Leu	Ser	Thr	Gly	Arg	Val	Gln	Gly	Tyr	Glu	Gly	Tyr	Tyr
1				5					10					15	

Val	Leu	Pro	Val	Glu	Gln	Tyr	Pro	Glu	Leu	Ala	Asp	Ser	Ser	Ser	Asn
			20					25					30		

Ile	Gln	Phe	Leu	Arg	Gln	Asn	Glu	Met
		35				40		

<210> 79

45/88

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP2 (43 a.a.)

<400> 79

Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr Tyr Val
 1 5 10 15

Leu Pro Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Asn Glu Met Gly Lys Arg
 35 40

<210> 80

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP2 (44 a.a.)

<400> 80

Gln Gln Leu Leu Ser Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr Tyr
 1 5 10 15

Val Leu Pro Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Asn Glu Met Gly Lys Arg
 35 40

<210> 81

<211> 120

<212> DNA

46/88

<213> Artificial Sequence

<220>

<223> Human TCAP2 (120 n.a.)

<400> 81
 cagcttctga gcaccgggcg cgtgcaaggg tacgagggat attacgtgct tcccgtggag 60
 caataccag agcttgcaga cagtagcagc aacatccagt ttttaagaca gaatgagatg 120

<210> 82

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP2 (123 n.a.)

<400> 82
 cagcagcttc tgagcaccgg gcgcgtgcaa ggttacgagg gatattacgt gcttcccgtg 60
 gagcaatacc cagagcttgc agacagtagc agcaacatcc agtttttaag acagaatgag 120
 atg 123

<210> 83

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP2 (129 n.a.)

<400> 83
 cagcttctga gcaccgggcg cgtgcaaggg tacgagggat attacgtgct tcccgtggag 60
 caataccag agcttgcaga cagtagcagc aacatccagt ttttaagaca gaatgagatg 120
 ggaaagagg 129

47/88

<210> 84

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP2 (132 n.a.)

<400> 84
cagcagcttc tgagcaccgg ggcgctgcaa ggttacgagg gatattacgt gcttcccgtg 60
gagcaatacc cagagcttgc agacagtagc agcaacatcc agtttttaag acagaatgag 120
atgggaaaga gg 132

<210> 85

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP3 (40 a.a.)

<400> 85

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
1 5 10 15

Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile
35 40

<210> 86

<211> 41

<212> PRT

<213> Artificial Sequence

48/88

<220>

<223> Human TCAP3 (41 a.a.)

<400> 86

Arg Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Ser Glu Ile
 35 40

<210> 87

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP3 (43 a.a.)

<400> 87

Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val
 1 5 10 15

Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile
 20 25 30

Gln Phe Leu Arg Gln Ser Glu Ile Gly Arg Arg
 35 40

<210> 88

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

49/88

<223> Human preTCAP3 (44 a.a.)

<400> 88

Arg Gln Leu Leu Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn
 20 25 30

Ile Gln Phe Leu Arg Gln Ser Glu Ile Gly Arg Arg
 35 40

<210> 89

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP3 (120 n.a.)

<400> 89
 cagctgctga gcgccggcaa ggtgcagggc tacgacgggt actacgtact ctcggtggag 60
 cagtaccccg agctggccga cagcgccaac aacatccagt tcctgcggca gagcgagatc 120

<210> 90

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP3 (123 n.a.)

<400> 90
 cggcagctgc tgagcgccgg caaggtgcag ggctacgacg ggtactacgt actctcgggtg 60
 gagcagtacc ccgagctggc cgacagcgcc aacaacatcc agttcctgcg gcagagcgag 120
 atc 123

<210> 91

50/88

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP (129 n.a.)

<400> 91
cagctgctga gcgccggcaa ggtgcagggc tacgacgggt actacgtact ctcggtggag 60
cagtaccccg agctggccga cagcgccaac aacatccagt tcctgctggca gagcgagatc 120
ggcaggagg 129

<210> 92

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP3 (132 n.a.)

<400> 92
cggcagctgc tgagcgccgg caaggtgcag ggctacgacg ggtactacgt actctcgggtg 60
gagcagtacc ccgagctggc cgacagcgcc aacaacatcc agttcctgcg gcagagcgag 120
atcggcagga gg 132

<210> 93

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP4 (40 a.a.)

<400> 93

51/88

Gln Val Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe Val
 1 5 10 15

Ile Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30

His Phe Met Arg Gln Ser Glu Met
 35 40

<210> 94

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Human TCAP4 (41 a.a.)

<400> 94

Gln Gln Val Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe
 1 5 10 15

Val Ile Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Met
 35 40

<210> 95

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP4 (43 a..a)

<400> 95

Gln Val Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe Val
 1 5 10 15

52/88

Ile Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn Ile
 20 25 30

His Phe Met Arg Gln Ser Glu Met Gly Arg Arg
 35 40

<210> 96

<211> 44

<212> PRT

<213> Artificial Sequence

<220>

<223> Human preTCAP4 (44 a.a.)

<400> 96

Gln Gln Val Leu Ser Thr Gly Arg Val Gln Gly Tyr Asp Gly Phe Phe
 1 5 10 15

Val Ile Ser Val Glu Gln Tyr Pro Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Met Gly Arg Arg
 35 40

<210> 97

<211> 120

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP4 (120 n.a.)

<400> 97
 caggtgctga gcacagggcg ggtgcaaggc tacgacggct ttttcgtgat ctctgtcgag 60

cagtaccag aactgtcaga cagcgccaac aacatccact tcatgagaca gagcgagatg 120

<210> 98

53/88

<211> 123

<212> DNA

<213> Artificial Sequence

<220>

<223> Human TCAP4 (123 n.a.)

<400> 98
cagcaggtgc tgagcacagg gcgggtgcaa ggctacgacg gctttttcgt gatctctgtc 60
gagcagtacc cagaactgtc agacagcgcc aacaacatcc acttcatgag acagagcgag 120
atg 123

<210> 99

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP4 (129 n.a.)

<400> 99
caggtgctga gcacagggcg ggtgcaaggc tacgacggct ttttcgtgat ctctgtcgag 60
cagtaccag aactgtcaga cagcgccaac aacatccact tcatgagaca gagcgagatg 120
ggccggagg 129

<210> 100

<211> 132

<212> DNA

<213> Artificial Sequence

<220>

<223> Human preTCAP4 (132 n.a.)

<400> 100
cagcaggtgc tgagcacagg gcgggtgcaa ggctacgacg gctttttcgt gatctctgtc 60

54/88

gagcagtacc cagaactgtc agacagcgcc aacaacatcc acttcatgag acagagcgag 120
 atgggccgga gg 132

<210> 101

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> G. gallus TCAP-1

<400> 101

Gln Gln Leu Leu Asn Thr Gly Arg Val Gln Gly Tyr Asp Gly Tyr Phe
 1 5 10 15

Val Leu Ser Val Glu Gln Tyr Leu Glu Leu Ser Asp Ser Ala Asn Asn
 20 25 30

Ile His Phe Met Arg Gln Ser Glu Ile
 35 40

<210> 102

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Zebrafish TCAP-4

<400> 102

Gln Gln Leu Leu Ser Ser Gly Arg Val Gln Gly Tyr Glu Gly Phe Tyr
 1 5 10 15

Ile Val Ser Val Asp Gln Phe Pro Glu Leu Thr Asp Asn Ile Asn Asn
 20 25 30

Val His Phe Trp Arg Gln Thr Glu Met
 35 40

55/88

<210> 103

<211> 37

<212> PRT

<213> Artificial Sequence

<220>

<223> D. melanogaster Ten-m gene product

<400> 103

Glu Leu Val Gln His Gly Asp Val Asp Gly Trp Asn Gly Asp Ile His
 1 5 10 15

Ser Ile His Lys Tyr Pro Gln Leu Ala Asp Pro Gly Asn Val Ala Phe
 20 25 30

Gln Arg Asp Ala Lys
 35

<210> 104

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> Human CRF TCAP like region

<400> 104

Ser Glu Glu Pro Pro Ile Ser Leu Asp Leu Thr Phe His Leu Leu Arg
 1 5 10 15

Glu Val Leu Glu Met Ala Arg Ala Glu Gln Leu Ala Gln Gln Ala His
 20 25 30

Ser Asn Arg Lys Leu Met Glu Ile Ile
 35 40

<210> 105

56/88

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> Human urocortin TCAP-like region

<400> 105

Asp	Asn	Pro	Ser	Leu	Ser	Ile	Asp	Leu	Thr	Phe	His	Leu	Leu	Arg	Thr
1				5					10					15	

Leu	Leu	Glu	Leu	Ala	Arg	Thr	Gln	Ser	Gln	Arg	Glu	Arg	Ala	Glu	Gln
			20					25					30		

Asn	Arg	Ile	Ile	Phe	Asp	Ser	Val
		35					40

<210> 106

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> Human urocortin 2 TCAP-like region

<400> 106

Ile	Val	Leu	Ser	Leu	Asp	Val	Pro	Ile	Gly	Leu	Leu	Gln	Ile	Leu	Leu
1				5					10					15	

Glu	Gln	Ala	Arg	Ala	Arg	Ala	Ala	Arg	Glu	Gln	Ala	Thr	Thr	Asn	Ala
			20					25					30		

Arg	Ile	Leu	Ala	Arg	Val
		35			

<210> 107

<211> 38

57/88

<212> PRT

<213> Artificial Sequence

<220>

<223> Human urocortin 3 TCAP=like region

<400> 107

Phe	Thr	Leu	Ser	Leu	Asp	Val	Pro	Thr	Asn	Ile	Met	Asn	Leu	Leu	Phe
1				5					10					15	

Asn	Ile	Ala	Lys	Ala	Lys	Asn	Leu	Arg	Ala	Gln	Ala	Ala	Ala	Asn	Ala
			20					25						30	

His	Leu	Met	Ala	Gln	Ile
		35			

<210> 108

<211> 46

<212> PRT

<213> Artificial Sequence

<220>

<223> L. migratoria DP

<400> 108

Met	Gly	Met	Gly	Pro	Ser	Leu	Ser	Ile	Val	Asn	Pro	Met	Asp	Val	Leu
1				5					10					15	

Arg	Gln	Arg	Leu	Leu	Leu	Glu	Ile	Ala	Arg	Arg	Arg	Leu	Arg	Asp	Ala
			20					25					30		

Glu	Glu	Gln	Ile	Lys	Ala	Asn	Lys	Asp	Phe	Leu	Gln	Gln	Ile
		35					40					45	

<210> 109

<211> 46

<212> PRT

<213> Artificial Sequence

58/88

<220>

<223> A. domesticus DP

<400> 109

Thr Gly Ala Gln Ser Leu Ser Ile Val Ala Pro Leu Asp Val Leu Arg
 1 5 10 15

Gln Arg Leu Met Asn Glu Leu Asn Arg Arg Arg Met Arg Glu Leu Gln
 20 25 30

Gly Ser Arg Ile Gln Gln Asn Arg Gln Leu Leu Thr Ser Ile
 35 40 45

<210> 110

<211> 39

<212> PRT

<213> Artificial Sequence

<220>

<223> T. molitor DP

<400> 110

Ser Pro Thr Ile Ser Ile Thr Ala Pro Ile Asp Val Leu Arg Lys Thr
 1 5 10 15

Trp Glu Gln Glu Arg Ala Arg Lys Gln Met Val Ala Gln Asn Asn Arg
 20 25 30

Glu Phe Leu Asn Ser Leu Asn
 35

<210> 111

<211> 41

<212> PRT

<213> Artificial Sequence

59/88

<220>

<223> M. sexta DP-1

<400> 111

Arg Met Pro Ser Leu Ser Ile Asp Leu Pro Met Ser Val Leu Arg Gln
 1 5 10 15

Lys Leu Ser Leu Glu Lys Glu Arg Lys Val His Ala Leu Arg Ala Ala
 20 25 30

Ala Asn Arg Asn Phe Leu Asn Asp Ile
 35 40

<210> 112

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> M. sexta DP-II

<400> 112

Ser Leu Ser Val Asn Pro Ala Val Asp Ile Leu Gln His Arg Tyr Met
 1 5 10 15

Glu Lys Val Ala Gln Asn Asn Arg Asn Phe Leu Asn Arg Val
 20 25 30

<210> 113

<211> 45

<212> PRT

<213> Artificial Sequence

<220>

<223> P. Americana

<400> 113

Thr Gly Ser Gly Pro Ser Leu Ser Ile Val Asn Pro Leu Asp Val Leu

61/88

Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln
 20 25 30

Asn Arg Ile Ile Phe Asp Ser Val
 35 40

<210> 116

<211> 37

<212> PRT

<213> Artificial Sequence

<220>

<223> P. sauvageii

<400> 116

Gln Gly Pro Pro Ile Ser Ile Asp Leu Ser Leu Glu Leu Leu Arg Lys
 1 5 10 15

Met Ile Glu Ile Glu Lys Gln Glu Lys Glu Lys Gln Gln Ala Ala Asn
 20 25 30

Asn Arg Leu Leu Leu
 35

<210> 117

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> C. carpio US

<400> 117

Asn Asp Asp Pro Pro Ile Ser Ile Asp Leu Thr Phe His Leu Leu Arg
 1 5 10 15

Asn Met Ile Glu Met Ala Arg Asn Glu Asn Gln Arg Glu Gln Ala Gly
 20 25 30

62/88

Leu Asn Arg Lys Tyr Leu Asp Glu Val
 35 40

<210> 118

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> M. Musculus UCN2

<400> 118

Val Ile Leu Ser Leu Asp Val Pro Ile Gly Leu Leu Arg Ile Leu Leu
 1 5 10 15

Glu Gln Ala Arg Tyr Lys Ala Ala Arg Asn Gln Ala Ala Thr Asn Ala
 20 25 30

Gln Ile Leu Ala His Val
 35

<210> 119

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> R. dano UCN2

<400> 119

Leu Thr Leu Ser Leu Asp Val Pro Thr Asn Ile Met Asn Val Leu Phe
 1 5 10 15

Asp Val Ala Lys Ala Lys Asn Leu Arg Ala Lys Ala Ala Glu Asn Ala
 20 25 30

Arg Leu Leu Ala His Ile
 35

63/88

<210> 120

<211> 305

<212> DNA

<213> Artificial Sequence

<220>

<223> Hamster 305bp urocortin cDNA probe examples "cloning mRNA"

<400> 120
 attcaccgcc gctcgggatc tgagcctgca ggcgagcggc agcgacggga agaccttccg 60
 ctgtccatcg acctcacatt ccacctgcta cggaccctgc tggagatggc ccggacacag 120
 agccaacgcg agcgagcaga gcagaaccga atcactactca acgcggtggg caagtgatcg 180
 gcccggtgtg ggacccccaaa aggctcgacc ctttccccta cctacccccg ggctgaagtc 240
 acgcgaccga agtcggctta gtcccgcggt gcagcgcctc ccagagttac cctgaacaat 300
 cccgc 305

<210> 121

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP1 fwd primer

<400> 121
 acgtcagtgt tgatgggagg acta 24

<210> 122

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

64/88

<223> TCAP1 rvs primer

<400> 122
cctcctgcct atttcactct gtctcat

27

<210> 123

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP2 Fwd primer

<400> 123
tcgagggcaa ggacacacac tactt

25

<210> 124

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP2 rvs primer

<400> 124
aagaactgga tgttgctgct actgtc

26

<210> 125

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP3 fwd primer

<400> 125
caacaacgcc ttctacctgg agaac

25

65/88

<210> 126

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP3 rvs primer

<400> 126

tgttgtggc actgtcagcc a

21

<210> 127

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP4 fwd primer

<400> 127

tttgctcca gtggttccat ctt

23

<210> 128

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> TCAP4 rvs primer

<400> 128

tggatattgt tggcgctgtc tgac

24

<210> 129

<211> 6

66/88

<212> PRT

<213> Artificial Sequence

<220>

<223> Conserved motif between CRF and TCAP I/L S X X (X)-L/V at amino terminus

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> X=I or L

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> X=T or A

<220>

<221> MISC_FEATURE

<222> (4)..(4)

<223> X=L, I or G

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> X=D, R or K

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> X=L or V

67/88

<400> 129

Xaa Ser Xaa Xaa Xaa Xaa
1 5

<210> 130

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Conserved motif between CRF and TCAP - In middle L/V-L/I-X-V/aliphatic residue

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> X=V or L

<220>

<221> MISC_FEATURE

<222> (4)..(4)

<223> X=M, L Q, I or V

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> X=L, I or F

<220>

<221> MISC_FEATURE

<222> (3)..(3)

68/88

<223> X=E, N, S or P

<400> 130

Xaa Xaa Xaa Xaa
1

<210> 131

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Conserved motif between CRF and TCAP N/I/A-H/basic residue -I/L/F
/-aliphatic at carboxy terminus

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> X=R, A or I

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> X=H or basic residues, K, I, R or Q

<220>

<221> MISC_FEATURE

<222> (4)..(4)

<223> X=I, L or F

<400> 131

Asn Xaa Xaa Xaa

69/88

1

<210> 132

<211> 8964

<212> DNA

<213> Mus musculus

<220>

<221> exon

<222> (50)..(8197)

<223>

<400> 132

aagttctaag aagccggacc gatgtgcaca gagaaggaat gaaggaagt atg gat gtg	58
Met Asp Val	
1	
aag gaa cgc agg cct tac tgc tcc ttg acc aag agc aga cgg gaa aag	106
Lys Glu Arg Arg Pro Tyr Cys Ser Leu Thr Lys Ser Arg Arg Glu Lys	
5 10 15	
gaa agg cgc tat aca aat tcg tcc gcg gac aat gag gag tgt agg gtc	154
Glu Arg Arg Tyr Thr Asn Ser Ser Ala Asp Asn Glu Glu Cys Arg Val	
20 25 30 35	
ccc acg cag aag tcc tat agt tcc agt gaa acc ttg aaa gct ttc gat	202
Pro Thr Gln Lys Ser Tyr Ser Ser Ser Glu Thr Leu Lys Ala Phe Asp	
40 45 50	
cat gat tat tca cgg ctg ctt tat gga aac aga gta aag gat ttg gtc	250
His Asp Tyr Ser Arg Leu Leu Tyr Gly Asn Arg Val Lys Asp Leu Val	
55 60 65	
cac aga gaa gcc gac gag tat act aga caa gga cag aat ttt acc cta	298
His Arg Glu Ala Asp Glu Tyr Thr Arg Gln Gly Gln Asn Phe Thr Leu	
70 75 80	
agg cag tta gga gtg tgt gaa tcc gca act cga aga gga gtg gca ttc	346
Arg Gln Leu Gly Val Cys Glu Ser Ala Thr Arg Arg Gly Val Ala Phe	
85 90 95	
tgt gcg gaa atg ggg ctc cct cac aga ggt tac tcc atc agt gca ggg	394
Cys Ala Glu Met Gly Leu Pro His Arg Gly Tyr Ser Ile Ser Ala Gly	
100 105 110 115	
tca gat gcg gat acg gaa aac gaa gca gtg atg tcc cct gag cat gcc	442
Ser Asp Ala Asp Thr Glu Asn Glu Ala Val Met Ser Pro Glu His Ala	
120 125 130	

70/88

atg aga ctt tgg ggc agg ggg gtc aaa tcg ggc cgc agt tcc tgc ctg	490
Met Arg Leu Trp Gly Arg Gly Val Lys Ser Gly Arg Ser Ser Cys Leu	
135 140 145	
tca agc cgg tcc aac tcc gcc ctc acc ctg aca gac acg gag cac gag	538
Ser Ser Arg Ser Asn Ser Ala Leu Thr Leu Thr Asp Thr Glu His Glu	
150 155 160	
aac agg tcg gac agt gag agc gag caa cct tca aac aac cca ggg caa	586
Asn Arg Ser Asp Ser Glu Ser Glu Gln Pro Ser Asn Asn Pro Gly Gln	
165 170 175	
ccc acc ctg cag cct ttg ccg cca tcc cac aag cag cac ccg gcg cag	634
Pro Thr Leu Gln Pro Leu Pro Pro Ser His Lys Gln His Pro Ala Gln	
180 185 190 195	
cat cac ccg tcc atc act tcc ctc aat aga aac tcc ctg acc aat aga	682
His His Pro Ser Ile Thr Ser Leu Asn Arg Asn Ser Leu Thr Asn Arg	
200 205 210	
agg aac cag agt ccg gcc ccg ccg gct gct ttg ccc gcc gag ctg caa	730
Arg Asn Gln Ser Pro Ala Pro Pro Ala Ala Leu Pro Ala Glu Leu Gln	
215 220 225	
acc aca ccc gag tcc gtc cag ctg cag gac agc tgg gtc ctt ggc agt	778
Thr Thr Pro Glu Ser Val Gln Leu Gln Asp Ser Trp Val Leu Gly Ser	
230 235 240	
aat gta cca ctg gaa agc agg cat ttc cta ttc aaa aca ggg aca ggg	826
Asn Val Pro Leu Glu Ser Arg His Phe Leu Phe Lys Thr Gly Thr Gly	
245 250 255	
acg acg cca ctg ttc agt acg gca acc ccg gga tac aca atg gca tct	874
Thr Thr Pro Leu Phe Ser Thr Ala Thr Pro Gly Tyr Thr Met Ala Ser	
260 265 270 275	
ggc tct gtt tat tct ccg cct acc cgg cca ctt cct aga aac acc cta	922
Gly Ser Val Tyr Ser Pro Pro Thr Arg Pro Leu Pro Arg Asn Thr Leu	
280 285 290	
tca aga agt gct ttt aaa ttc aag aag tct tca aag tac tgc agc tgg	970
Ser Arg Ser Ala Phe Lys Phe Lys Lys Ser Ser Lys Tyr Cys Ser Trp	
295 300 305	
agg tgc acc gca ctg tgt gct gta ggg gtc tca gtg ctc ctg gcc att	1018
Arg Cys Thr Ala Leu Cys Ala Val Gly Val Ser Val Leu Leu Ala Ile	
310 315 320	
ctc ctc tcc tat ttt ata gca atg cat cta ttt ggc ctc aac tgg cac	1066
Leu Leu Ser Tyr Phe Ile Ala Met His Leu Phe Gly Leu Asn Trp His	
325 330 335	
tta cag cag acg gaa aat gac aca ttc gag aat gga aaa gtg aat tct	1114
Leu Gln Gln Thr Glu Asn Asp Thr Phe Glu Asn Gly Lys Val Asn Ser	
340 345 350 355	
gac acc gtg cca aca aac act gta tcg tta cct tct ggc gac aat gga	1162
Asp Thr Val Pro Thr Asn Thr Val Ser Leu Pro Ser Gly Asp Asn Gly	

71/88

			360				365				370					
aaa	tta	ggt	gga	ttt	aca	cat	gaa	aat	aac	acc	ata	gat	tcc	gga	gaa	1210
Lys	Leu	Gly	Gly	Phe	Thr	His	Glu	Asn	Asn	Thr	Ile	Asp	Ser	Gly	Glu	
			375				380				385					
ctt	gat	att	ggc	cgg	aga	gca	att	caa	gag	gtt	ccc	ccc	ggg	atc	ttc	1258
Leu	Asp	Ile	Gly	Arg	Arg	Ala	Ile	Gln	Glu	Val	Pro	Pro	Gly	Ile	Phe	
			390				395				400					
tgg	aga	tcg	cag	ctc	ttt	att	gat	cag	cca	cag	ttt	ctt	aag	ttc	aac	1306
Trp	Arg	Ser	Gln	Leu	Phe	Ile	Asp	Gln	Pro	Gln	Phe	Leu	Lys	Phe	Asn	
			405				410				415					
atc	tct	ctt	cag	aag	gat	gca	ttg	atc	gga	gtg	tac	ggc	cgg	aag	ggc	1354
Ile	Ser	Leu	Gln	Lys	Asp	Ala	Leu	Ile	Gly	Val	Tyr	Gly	Arg	Lys	Gly	
			420				425				430				435	
tta	ccg	cct	tcc	cat	act	cag	tac	gac	ttt	gtg	gaa	cta	ctg	gat	ggt	1402
Leu	Pro	Pro	Ser	His	Thr	Gln	Tyr	Asp	Phe	Val	Glu	Leu	Leu	Asp	Gly	
			440				445				450					
agc	agg	tta	att	gcg	aga	gag	cag	cgg	aac	ctg	gtg	gag	tcc	gaa	aga	1450
Ser	Arg	Leu	Ile	Ala	Arg	Glu	Gln	Arg	Asn	Leu	Val	Glu	Ser	Glu	Arg	
			455				460				465					
gcc	ggg	cgg	cag	gcg	aga	tct	gtc	agc	ctg	cac	gaa	gct	ggc	ttc	atc	1498
Ala	Gly	Arg	Gln	Ala	Arg	Ser	Val	Ser	Leu	His	Glu	Ala	Gly	Phe	Ile	
			470				475				480					
cag	tac	ttg	gat	tct	gga	atc	tgg	cat	ctg	gct	ttt	tat	aac	gac	ggg	1546
Gln	Tyr	Leu	Asp	Ser	Gly	Ile	Trp	His	Leu	Ala	Phe	Tyr	Asn	Asp	Gly	
			485				490				495					
aaa	aac	cca	gag	cag	gtc	tcc	ttt	aac	acg	atc	gtt	ata	gag	tct	gtg	1594
Lys	Asn	Pro	Glu	Gln	Val	Ser	Phe	Asn	Thr	Ile	Val	Ile	Glu	Ser	Val	
			500				505				510				515	
gtg	gaa	tgc	ccc	cga	aat	tgc	cat	gga	aat	gga	gag	tgt	gtt	tct	gga	1642
Val	Glu	Cys	Pro	Arg	Asn	Cys	His	Gly	Asn	Gly	Glu	Cys	Val	Ser	Gly	
			520				525				530					
act	tgc	cat	tgt	ttc	ccc	ggg	ttt	cta	ggt	ccg	gat	tgt	tca	aga	gca	1690
Thr	Cys	His	Cys	Phe	Pro	Gly	Phe	Leu	Gly	Pro	Asp	Cys	Ser	Arg	Ala	
			535				540				545					
gcc	tgt	ccg	gtg	ctc	tgt	agt	ggc	aac	ggg	caa	tac	tcc	aag	ggc	cgc	1738
Ala	Cys	Pro	Val	Leu	Cys	Ser	Gly	Asn	Gly	Gln	Tyr	Ser	Lys	Gly	Arg	
			550				555				560					
tgc	ctg	tgc	ttc	agt	ggc	tgg	aag	ggc	acc	gag	tgt	gac	gtg	ccg	acg	1786
Cys	Leu	Cys	Phe	Ser	Gly	Trp	Lys	Gly	Thr	Glu	Cys	Asp	Val	Pro	Thr	
			565				570				575					
acc	cag	tgc	att	gac	ccg	cag	tgc	ggg	ggt	cgt	ggg	att	tgc	atc	atg	1834
Thr	Gln	Cys	Ile	Asp	Pro	Gln	Cys	Gly	Gly	Arg	Gly	Ile	Cys	Ile	Met	
			580				585				590				595	
ggc	tct	tgc	gct	tgt	aac	tcg	gga	tac	aaa	gga	gaa	aac	tgt	gag	gaa	1882

72/88

Gly	Ser	Cys	Ala	Cys	Asn	Ser	Gly	Tyr	Lys	Gly	Glu	Asn	Cys	Glu	Glu		
				600					605					610			
gcg	gac	tgt	cta	gac	cct	gga	tgt	tct	aat	cac	ggg	gtg	tgt	atc	cat		1930
Ala	Asp	Cys	Leu	Asp	Pro	Gly	Cys	Ser	Asn	His	Gly	Val	Cys	Ile	His		
			615					620					625				
ggg	gaa	tgt	cac	tgc	aat	cca	ggc	tgg	ggt	ggc	agc	aac	tgt	gaa	ata		1978
Gly	Glu	Cys	His	Cys	Asn	Pro	Gly	Trp	Gly	Gly	Ser	Asn	Cys	Glu	Ile		
		630					635					640					
ctg	aag	act	atg	tgt	gca	gac	cag	tgc	tca	ggc	cac	ggg	act	tac	ctt		2026
Leu	Lys	Thr	Met	Cys	Ala	Asp	Gln	Cys	Ser	Gly	His	Gly	Thr	Tyr	Leu		
	645					650					655						
caa	gaa	agc	ggc	tcc	tgc	act	tgc	gac	cca	aat	tgg	act	ggc	ccc	gac		2074
Gln	Glu	Ser	Gly	Ser	Cys	Thr	Cys	Asp	Pro	Asn	Trp	Thr	Gly	Pro	Asp		
660					665					670					675		
tgc	tca	aat	gaa	ata	tgt	tca	gtg	gac	tgc	ggc	tca	cac	ggc	gtc	tgc		2122
Cys	Ser	Asn	Glu	Ile	Cys	Ser	Val	Asp	Cys	Gly	Ser	His	Gly	Val	Cys		
				680					685					690			
atg	ggg	ggc	tcc	tgt	cgc	tgt	gaa	gaa	ggc	tgg	acc	ggc	ccg	gcg	tgt		2170
Met	Gly	Gly	Ser	Cys	Arg	Cys	Glu	Glu	Gly	Trp	Thr	Gly	Pro	Ala	Cys		
			695					700					705				
aat	cag	aga	gct	tgc	cac	cct	cgc	tgt	gct	gag	cac	ggg	acg	tgc	aag		2218
Asn	Gln	Arg	Ala	Cys	His	Pro	Arg	Cys	Ala	Glu	His	Gly	Thr	Cys	Lys		
		710					715					720					
gac	ggc	aag	tgc	gag	tgc	agc	caa	gga	tgg	aac	gga	gag	cac	tgc	aca		2266
Asp	Gly	Lys	Cys	Glu	Cys	Ser	Gln	Gly	Trp	Asn	Gly	Glu	His	Cys	Thr		
	725					730					735						
att	gct	cac	tat	ttg	gat	aag	ata	gtt	aaa	gag	ggt	tgc	ccc	ggc	ttg		2314
Ile	Ala	His	Tyr	Leu	Asp	Lys	Ile	Val	Lys	Glu	Gly	Cys	Pro	Gly	Leu		
740					745					750					755		
tgc	aac	agc	aat	ggg	aga	tgc	aca	ctg	gac	caa	aac	ggc	tgg	cac	tgc		2362
Cys	Asn	Ser	Asn	Gly	Arg	Cys	Thr	Leu	Asp	Gln	Asn	Gly	Trp	His	Cys		
				760					765					770			
gtt	tgc	cag	cca	ggg	tgg	aga	gga	gca	ggc	tgt	gac	gta	gcc	atg	gag		2410
Val	Cys	Gln	Pro	Gly	Trp	Arg	Gly	Ala	Gly	Cys	Asp	Val	Ala	Met	Glu		
			775					780					785				
acc	ctc	tgt	aca	gac	agc	aaa	gac	aac	gaa	gga	gac	gga	ctc	att	gac		2458
Thr	Leu	Cys	Thr	Asp	Ser	Lys	Asp	Asn	Glu	Gly	Asp	Gly	Leu	Ile	Asp		
		790					795					800					
tgc	atg	gat	cct	gat	tgc	tgc	ctc	cag	agc	tcc	tgc	caa	aac	cag	ccc		2506
Cys	Met	Asp	Pro	Asp	Cys	Cys	Leu	Gln	Ser	Ser	Cys	Gln	Asn	Gln	Pro		
	805					810						815					
tac	tgt	cgt	ggc	ttg	cct	gat	cct	cag	gat	atc	att	agc	caa	agc	ctt		2554
Tyr	Cys	Arg	Gly	Leu	Pro	Asp	Pro	Gln	Asp	Ile	Ile	Ser	Gln	Ser	Leu		
820					825					830					835		

73/88

cag aca cca tct cag caa gct gcc aag tcc ttc tat gac cga atc agt Gln Thr Pro Ser Gln Gln Ala Ala Lys Ser Phe Tyr Asp Arg Ile Ser 840 845 850	2602
ttc ctg att gga tcg gat agc acc cac gtg ctc cct gga gaa agt ccg Phe Leu Ile Gly Ser Asp Ser Thr His Val Leu Pro Gly Glu Ser Pro 855 860 865	2650
ttc aat aag agt ctt gcg tcc gtc atc aga ggc caa gta cta aca gct Phe Asn Lys Ser Leu Ala Ser Val Ile Arg Gly Gln Val Leu Thr Ala 870 875 880	2698
gat gga acc cca ctt att ggc gtc aac gtg tcg ttt tta cac tac tcg Asp Gly Thr Pro Leu Ile Gly Val Asn Val Ser Phe Leu His Tyr Ser 885 890 895	2746
gaa tat gga tat acc att acc cgc cag gat gga atg ttt gac ttg gtg Glu Tyr Gly Tyr Thr Ile Thr Arg Gln Asp Gly Met Phe Asp Leu Val 900 905 910 915	2794
gca aat ggt ggc gct tct ctg act ttg gta ttt gag cgt tcc cca ttc Ala Asn Gly Gly Ala Ser Leu Thr Leu Val Phe Glu Arg Ser Pro Phe 920 925 930	2842
ctc act cag tac cac act gtg tgg att ccc tgg aat gtc ttt tat gtg Leu Thr Gln Tyr His Thr Val Trp Ile Pro Trp Asn Val Phe Tyr Val 935 940 945	2890
atg gat acc ctt gtc atg aag aaa gag gag aac gac att ccc agc tgt Met Asp Thr Leu Val Met Lys Lys Glu Glu Asn Asp Ile Pro Ser Cys 950 955 960	2938
gac ctc agt ggc ttt gtg agg cca agt ccc atc att gtg tct tca ccg Asp Leu Ser Gly Phe Val Arg Pro Ser Pro Ile Ile Val Ser Ser Pro 965 970 975	2986
tta tcc acc ttc ttc agg tct tcc cct gag gac agc ccc atc atc ccc Leu Ser Thr Phe Phe Arg Ser Ser Pro Glu Asp Ser Pro Ile Ile Pro 980 985 990 995	3034
gag aca cag gtc ctg cat gaa gaa acc aca att cca gga aca gat Glu Thr Gln Val Leu His Glu Glu Thr Thr Ile Pro Gly Thr Asp 1000 1005 1010	3079
ttg aaa ctt tcc tac ctg agt tcc aga gcg gca ggg tac aag tca Leu Lys Leu Ser Tyr Leu Ser Ser Arg Ala Ala Gly Tyr Lys Ser 1015 1020 1025	3124
gtt ctt aag att acc atg acc cag gcc gtc ata ccg ttt aac ctc Val Leu Lys Ile Thr Met Thr Gln Ala Val Ile Pro Phe Asn Leu 1030 1035 1040	3169
atg aag gtc cat ctg atg gtg gcc gtg gtt ggg aga ctc ttc cag Met Lys Val His Leu Met Val Ala Val Val Gly Arg Leu Phe Gln 1045 1050 1055	3214
aag tgg ttt cct gcc tcg cca aac ttg gcc tac acg ttc atc tgg Lys Trp Phe Pro Ala Ser Pro Asn Leu Ala Tyr Thr Phe Ile Trp 1060 1065 1070	3259

74/88

gat aag acg gac gca	tat aat cag aaa gtc	tac ggc ttg tca gag	3304
Asp Lys Thr Asp Ala	Tyr Asn Gln Lys Val	Tyr Gly Leu Ser Glu	
	1075	1080	1085
gca gtt gtg tcc gtc	gga tac gag tac gag	tcg tgc ttg gac ctg	3349
Ala Val Val Ser Val	Gly Tyr Glu Tyr Glu	Ser Cys Leu Asp Leu	
	1090	1095	1100
act ctc tgg gaa aag	agg act gcc gtt ttg	caa ggc tat gag ttg	3394
Thr Leu Trp Glu Lys	Arg Thr Ala Val Leu	Gln Gly Tyr Glu Leu	
	1105	1110	1115
gat gct tcg aac atg	ggc ggc tgg acg ttg	gac aag cac cat gta	3439
Asp Ala Ser Asn Met	Gly Gly Trp Thr Leu	Asp Lys His His Val	
	1120	1125	1130
ctg gac gtt cag aac	ggt ata cta tac aaa	gga aat gga gaa aat	3484
Leu Asp Val Gln Asn	Gly Ile Leu Tyr Lys	Gly Asn Gly Glu Asn	
	1135	1140	1145
cag ttc atc tct cag	cag cct ccg gtg gtc	agc agc atc atg ggt	3529
Gln Phe Ile Ser Gln	Gln Pro Pro Val Val	Ser Ser Ile Met Gly	
	1150	1155	1160
aat ggt cgg agg cgt	agc atc tca tgc cca	agt tgc aat ggt caa	3574
Asn Gly Arg Arg Arg	Ser Ile Ser Cys Pro	Ser Cys Asn Gly Gln	
	1165	1170	1175
gct gac ggg aac aaa	ctc ctg gca ccc gtg	gcg ctt gcc tgt ggg	3619
Ala Asp Gly Asn Lys	Leu Leu Ala Pro Val	Ala Leu Ala Cys Gly	
	1180	1185	1190
atc gac ggc agt cta	tac gta ggg gat ttc	aat tac gtc cgg cgg	3664
Ile Asp Gly Ser Leu	Tyr Val Gly Asp Phe	Asn Tyr Val Arg Arg	
	1195	1200	1205
ata ttc ccg tct ggg	aat gtg aca agt gtt	tta gaa cta aga aat	3709
Ile Phe Pro Ser Gly	Asn Val Thr Ser Val	Leu Glu Leu Arg Asn	
	1210	1215	1220
aaa gat ttt aga cat	agt agc aac cca gct	cac aga tac tac ctg	3754
Lys Asp Phe Arg His	Ser Ser Asn Pro Ala	His Arg Tyr Tyr Leu	
	1225	1230	1235
gct acg gac cca gtc	acc gga gat ttg tac	gtc tct gat act aac	3799
Ala Thr Asp Pro Val	Thr Gly Asp Leu Tyr	Val Ser Asp Thr Asn	
	1240	1245	1250
acc cgc aga atc tat	cgg ccg aaa tca ctc	acg gga gcc aaa gac	3844
Thr Arg Arg Ile Tyr	Arg Pro Lys Ser Leu	Thr Gly Ala Lys Asp	
	1255	1260	1265
ctg act aaa aac gct	gaa gtg gtg gca ggg	acc ggg gaa cag tgc	3889
Leu Thr Lys Asn Ala	Glu Val Val Ala Gly	Thr Gly Glu Gln Cys	
	1270	1275	1280
ctt ccc ttt gac gag	gcc agg tgt ggg gat	gga ggc aag gct gtg	3934
Leu Pro Phe Asp Glu	Ala Arg Cys Gly Asp	Gly Gly Lys Ala Val	

75/88

1285					1290					1295					
gaa	gca	acg	ctc	atg	agt	ccc	aaa	gga	atg	gca	atc	gat	aag	aac	3979
Glu	Ala	Thr	Leu	Met	Ser	Pro	Lys	Gly	Met	Ala	Ile	Asp	Lys	Asn	
				1300					1305					1310	
gga	ctg	atc	tac	ttt	gtt	gat	gga	acc	atg	atc	aga	aag	gtt	gat	4024
Gly	Leu	Ile	Tyr	Phe	Val	Asp	Gly	Thr	Met	Ile	Arg	Lys	Val	Asp	
				1315					1320					1325	
caa	aat	gga	atc	ata	tca	act	ctc	ctg	ggc	tcc	aac	gac	ctc	acg	4069
Gln	Asn	Gly	Ile	Ile	Ser	Thr	Leu	Leu	Gly	Ser	Asn	Asp	Leu	Thr	
				1330					1335					1340	
tca	gct	cga	cct	tta	acc	tgt	gat	act	agc	atg	cat	atc	agc	cag	4114
Ser	Ala	Arg	Pro	Leu	Thr	Cys	Asp	Thr	Ser	Met	His	Ile	Ser	Gln	
				1345					1350					1355	
gtg	cgt	ctg	gaa	tgg	ccc	act	gac	ctc	gcg	atc	aac	ccc	atg	gat	4159
Val	Arg	Leu	Glu	Trp	Pro	Thr	Asp	Leu	Ala	Ile	Asn	Pro	Met	Asp	
				1360					1365					1370	
aac	tcc	atc	tac	gtc	ctg	gat	aat	aac	gta	gtt	tta	cag	atc	act	4204
Asn	Ser	Ile	Tyr	Val	Leu	Asp	Asn	Asn	Val	Val	Leu	Gln	Ile	Thr	
				1375					1380					1385	
gaa	aac	cgt	cag	gtc	cgc	atc	gct	gcc	ggg	cgg	ccc	atg	cac	tgt	4249
Glu	Asn	Arg	Gln	Val	Arg	Ile	Ala	Ala	Gly	Arg	Pro	Met	His	Cys	
				1390					1395					1400	
cag	gtc	cct	gga	gtg	gaa	tac	ccg	gtg	ggg	aag	cac	gcg	gtt	cag	4294
Gln	Val	Pro	Gly	Val	Glu	Tyr	Pro	Val	Gly	Lys	His	Ala	Val	Gln	
				1405					1410					1415	
acc	acc	ctg	gag	tca	gcc	acg	gcc	att	gct	gtg	tcc	tac	agc	ggg	4339
Thr	Thr	Leu	Glu	Ser	Ala	Thr	Ala	Ile	Ala	Val	Ser	Tyr	Ser	Gly	
				1420					1425					1430	
gtc	ctt	tac	atc	acg	gaa	act	gat	gag	aag	aag	atc	aac	cga	ata	4384
Val	Leu	Tyr	Ile	Thr	Glu	Thr	Asp	Glu	Lys	Lys	Ile	Asn	Arg	Ile	
				1435					1440					1445	
agg	cag	gtc	acg	aca	gac	ggg	gag	atc	tcc	tta	gtg	gct	ggg	ata	4429
Arg	Gln	Val	Thr	Thr	Asp	Gly	Glu	Ile	Ser	Leu	Val	Ala	Gly	Ile	
				1450					1455					1460	
cct	tcg	gaa	tgt	gac	tgc	aag	aac	gac	gcc	aac	tgt	gac	tgc	tac	4474
Pro	Ser	Glu	Cys	Asp	Cys	Lys	Asn	Asp	Ala	Asn	Cys	Asp	Cys	Tyr	
				1465					1470					1475	
caa	agc	gga	gac	ggc	tac	gcc	aaa	gat	gcc	aaa	ctc	aat	gcg	ccg	4519
Gln	Ser	Gly	Asp	Gly	Tyr	Ala	Lys	Asp	Ala	Lys	Leu	Asn	Ala	Pro	
				1480					1485					1490	
tcc	tcc	ctg	gcc	gcc	tcg	cca	gat	ggc	act	ctg	tac	att	gca	gat	4564
Ser	Ser	Leu	Ala	Ala	Ser	Pro	Asp	Gly	Thr	Leu	Tyr	Ile	Ala	Asp	
				1495					1500					1505	
ctg	gga	aat	atc	agg	atc	cgg	gcc	gtt	tcg	aag	aat	aaa	cct	tta	4609

76/88

Leu	Gly	Asn	Ile	Arg	Ile	Arg	Ala	Val	Ser	Lys	Asn	Lys	Pro	Leu		
				1510					1515					1520		
ctg	aac	tca	atg	aac	ttt	tac	gaa	ggt	gcc	tct	cca	act	gat	caa		4654
Leu	Asn	Ser	Met	Asn	Phe	Tyr	Glu	Val	Ala	Ser	Pro	Thr	Asp	Gln		
				1525					1530					1535		
gag	ctc	tac	atc	ttt	gac	atc	aac	ggt	act	cac	cag	tac	acc	gtg		4699
Glu	Leu	Tyr	Ile	Phe	Asp	Ile	Asn	Gly	Thr	His	Gln	Tyr	Thr	Val		
				1540					1545					1550		
agc	ctg	gtc	acg	ggt	gac	tac	cta	tat	aat	ttt	agt	tac	agc	aat		4744
Ser	Leu	Val	Thr	Gly	Asp	Tyr	Leu	Tyr	Asn	Phe	Ser	Tyr	Ser	Asn		
				1555					1560					1565		
gac	aat	gac	gtc	acc	gct	gta	act	gac	agc	aat	ggc	aac	acc	ctc		4789
Asp	Asn	Asp	Val	Thr	Ala	Val	Thr	Asp	Ser	Asn	Gly	Asn	Thr	Leu		
				1570					1575					1580		
cga	atc	cga	agg	gat	ccg	aat	cgg	atg	ccg	gtg	cgg	gtg	gtg	tct		4834
Arg	Ile	Arg	Arg	Asp	Pro	Asn	Arg	Met	Pro	Val	Arg	Val	Val	Ser		
				1585					1590					1595		
cct	gat	aac	cag	gtg	ata	tgg	ttg	acc	ata	ggc	acc	aac	ggg	tgt		4879
Pro	Asp	Asn	Gln	Val	Ile	Trp	Leu	Thr	Ile	Gly	Thr	Asn	Gly	Cys		
				1600					1605					1610		
ctg	aaa	agc	atg	acc	gct	cag	ggc	ctg	gaa	ctg	ggt	ttg	ttt	act		4924
Leu	Lys	Ser	Met	Thr	Ala	Gln	Gly	Leu	Glu	Leu	Val	Leu	Phe	Thr		
				1615					1620					1625		
tac	cat	ggc	aac	agt	ggg	ctt	tta	gcc	acc	aaa	agt	gac	gaa	act		4969
Tyr	His	Gly	Asn	Ser	Gly	Leu	Leu	Ala	Thr	Lys	Ser	Asp	Glu	Thr		
				1630					1635					1640		
gga	tgg	aca	aca	ttt	ttt	gac	tat	gac	agt	gaa	ggt	cgc	ctg	acg		5014
Gly	Trp	Thr	Thr	Phe	Phe	Asp	Tyr	Asp	Ser	Glu	Gly	Arg	Leu	Thr		
				1645					1650					1655		
aat	ggt	acc	ttc	ccc	act	ggg	gtg	ggt	aca	aac	ctg	cac	ggg	gac		5059
Asn	Val	Thr	Phe	Pro	Thr	Gly	Val	Val	Thr	Asn	Leu	His	Gly	Asp		
				1660					1665					1670		
atg	gac	aag	gct	atc	acg	gtg	gac	atc	gag	tca	tcc	agc	aga	gag		5104
Met	Asp	Lys	Ala	Ile	Thr	Val	Asp	Ile	Glu	Ser	Ser	Ser	Arg	Glu		
				1675					1680					1685		
gaa	gat	gtc	agc	atc	act	tcg	aac	ttg	tcc	tcc	atc	gat	tcc	ttc		5149
Glu	Asp	Val	Ser	Ile	Thr	Ser	Asn	Leu	Ser	Ser	Ile	Asp	Ser	Phe		
				1690					1695					1700		
tac	acc	atg	gtc	caa	gac	cag	tta	aga	aac	agt	tac	cag	att	ggg		5194
Tyr	Thr	Met	Val	Gln	Asp	Gln	Leu	Arg	Asn	Ser	Tyr	Gln	Ile	Gly		
				1705					1710					1715		
tat	gat	ggc	tcc	ctt	aga	atc	ttc	tat	gcc	agt	ggt	ctg	gac	tct		5239
Tyr	Asp	Gly	Ser	Leu	Arg	Ile	Phe	Tyr	Ala	Ser	Gly	Leu	Asp	Ser		
				1720					1725					1730		

77/88

cac tac cag aca gag ccc cac gtt ctg gct ggc acg gcg aat ccc	5284
His Tyr Gln Thr Glu Pro His Val Leu Ala Gly Thr Ala Asn Pro	
1735 1740 1745	
aca gta gcc aaa aga aac atg act ctt ccc ggt gag aac ggg cag	5329
Thr Val Ala Lys Arg Asn Met Thr Leu Pro Gly Glu Asn Gly Gln	
1750 1755 1760	
aat ctg gtg gag tgg aga ttc cga aaa gaa caa gcc cag ggc aaa	5374
Asn Leu Val Glu Trp Arg Phe Arg Lys Glu Gln Ala Gln Gly Lys	
1765 1770 1775	
gtc aac gta ttc ggc cgg aag ctc agg gtc aat ggg cgc aac cta	5419
Val Asn Val Phe Gly Arg Lys Leu Arg Val Asn Gly Arg Asn Leu	
1780 1785 1790	
ctc tca gtg gac ttt gat cgg acc acc aag acg gaa aag atc tat	5464
Leu Ser Val Asp Phe Asp Arg Thr Thr Lys Thr Glu Lys Ile Tyr	
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gat gac cac cgg aaa ttt ctc ctg agg atc gct tac gac acg tcg	5509
Asp Asp His Arg Lys Phe Leu Leu Arg Ile Ala Tyr Asp Thr Ser	
1810 1815 1820	
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Gly His Pro Thr Leu Trp Leu Pro Ser Ser Lys Leu Met Ala Val	
1825 1830 1835	
aac gtc acc tac tca tcc acc ggt caa att gcc agc atc cag aga	5599
Asn Val Thr Tyr Ser Ser Thr Gly Gln Ile Ala Ser Ile Gln Arg	
1840 1845 1850	
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Gly Thr Thr Ser Glu Lys Val Asp Tyr Asp Ser Gln Gly Arg Ile	
1855 1860 1865	
gta tct cgg gtc ttt gcc gat ggg aaa aca tgg agt tac acg tac	5689
Val Ser Arg Val Phe Ala Asp Gly Lys Thr Trp Ser Tyr Thr Tyr	
1870 1875 1880	
ttg gaa aag tcc atg gtt ctt ctg ctc cat agc cag cgg cag tac	5734
Leu Glu Lys Ser Met Val Leu Leu Leu His Ser Gln Arg Gln Tyr	
1885 1890 1895	
atc ttc gaa tac gac atg tgg gac cgc ctg tcc gcc atc acc atg	5779
Ile Phe Glu Tyr Asp Met Trp Asp Arg Leu Ser Ala Ile Thr Met	
1900 1905 1910	
ccc agt gtg gct cgc cac acc atg cag acc atc cgg tcc att ggc	5824
Pro Ser Val Ala Arg His Thr Met Gln Thr Ile Arg Ser Ile Gly	
1915 1920 1925	
tac tac cgc aac atc tac aat ccc cca gaa agc aat gcc tct atc	5869
Tyr Tyr Arg Asn Ile Tyr Asn Pro Pro Glu Ser Asn Ala Ser Ile	
1930 1935 1940	
atc acc gac tac aac gag gaa ggg ctg ctt ctg caa aca gct ttc	5914
Ile Thr Asp Tyr Asn Glu Glu Gly Leu Leu Leu Gln Thr Ala Phe	
1945 1950 1955	

78/88

ctg gga acg agt cgg agg gtc tta ttc aag tat aga agg cag acc	5959
Leu Gly Thr Ser Arg Arg Val Leu Phe Lys Tyr Arg Arg Gln Thr	
1960 1965 1970	
agg cta tca gaa att tta tac gac agc aca aga gtc agt ttt acc	6004
Arg Leu Ser Glu Ile Leu Tyr Asp Ser Thr Arg Val Ser Phe Thr	
1975 1980 1985	
tac gac gaa aca gcg gga gtc ctg aaa aca gta aac ctt cag agt	6049
Tyr Asp Glu Thr Ala Gly Val Leu Lys Thr Val Asn Leu Gln Ser	
1990 1995 2000	
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Asp Gly Phe Ile Cys Thr Ile Arg Tyr Arg Gln Ile Gly Pro Leu	
2005 2010 2015	
att gac aga cag att ttc cgc ttc agc gag gat gga atg gta aat	6139
Ile Asp Arg Gln Ile Phe Arg Phe Ser Glu Asp Gly Met Val Asn	
2020 2025 2030	
gcg aga ttt gac tat agc tac gac aac agc ttt cga gtg acc agc	6184
Ala Arg Phe Asp Tyr Ser Tyr Asp Asn Ser Phe Arg Val Thr Ser	
2035 2040 2045	
atg cag ggt gtc atc aat gaa aca cca ctg ccc att gat cta tac	6229
Met Gln Gly Val Ile Asn Glu Thr Pro Leu Pro Ile Asp Leu Tyr	
2050 2055 2060	
cag ttt gat gac atc tct ggc aaa gtc gag cag ttt gga aaa ttc	6274
Gln Phe Asp Asp Ile Ser Gly Lys Val Glu Gln Phe Gly Lys Phe	
2065 2070 2075	
gga gtg ata tac tac gac atc aac caa atc att tcc acg gcc gtg	6319
Gly Val Ile Tyr Tyr Asp Ile Asn Gln Ile Ile Ser Thr Ala Val	
2080 2085 2090	
atg act tat aca aag cac ttt gat gct cat ggg cgc atc aag gag	6364
Met Thr Tyr Thr Lys His Phe Asp Ala His Gly Arg Ile Lys Glu	
2095 2100 2105	
atc caa tat gag ata ttt agg tca ctc atg tac tgg att aca att	6409
Ile Gln Tyr Glu Ile Phe Arg Ser Leu Met Tyr Trp Ile Thr Ile	
2110 2115 2120	
caa tat gat aat atg ggc cgg gta acc aag aga gag att aaa att	6454
Gln Tyr Asp Asn Met Gly Arg Val Thr Lys Arg Glu Ile Lys Ile	
2125 2130 2135	
ggg cct ttt gcc aac act acc aaa tac gcg tac gag tac gac gtc	6499
Gly Pro Phe Ala Asn Thr Thr Lys Tyr Ala Tyr Glu Tyr Asp Val	
2140 2145 2150	
gat gga cag ctc caa aca gtt tac cta aac gaa aag atc atg tgg	6544
Asp Gly Gln Leu Gln Thr Val Tyr Leu Asn Glu Lys Ile Met Trp	
2155 2160 2165	
cgg tac aac tac gac cta aat gga aac ctc cac ttg ctc aac ccc	6589
Arg Tyr Asn Tyr Asp Leu Asn Gly Asn Leu His Leu Leu Asn Pro	

79/88

2170	2175	2180	
agc agc agc gcc cgc ctg acc cct ctg cgc tat gac ctg cgc gac Ser Ser Ser Ala Arg Leu Thr Pro Leu Arg Tyr Asp Leu Arg Asp 2185 2190 2195			6634
aga atc acc cgc ctg ggc gat gtt cag tac cgg ctg gat gaa gat Arg Ile Thr Arg Leu Gly Asp Val Gln Tyr Arg Leu Asp Glu Asp 2200 2205 2210			6679
ggt ttc ctg cgt cag agg ggc act gaa att ttt gaa tac agc tcc Gly Phe Leu Arg Gln Arg Gly Thr Glu Ile Phe Glu Tyr Ser Ser 2215 2220 2225			6724
aaa ggg ctt ctg act cga gtc tac agt aaa ggc agt ggc tgg aca Lys Gly Leu Leu Thr Arg Val Tyr Ser Lys Gly Ser Gly Trp Thr 2230 2235 2240			6769
gtg atc tat cgg tac gac ggc ctg gga aga cgt gtt tct agc aaa Val Ile Tyr Arg Tyr Asp Gly Leu Gly Arg Arg Val Ser Ser Lys 2245 2250 2255			6814
acc agc ctg gga cag cac ctt cag ttt ttc tac gcc gac ctg aca Thr Ser Leu Gly Gln His Leu Gln Phe Phe Tyr Ala Asp Leu Thr 2260 2265 2270			6859
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atc acc tcc ctg tac tat gac ctc caa gga cat ctc ttc gcc atg Ile Thr Ser Leu Tyr Tyr Asp Leu Gln Gly His Leu Phe Ala Met 2290 2295 2300			6949
gag atc agc agt ggg gat gag ttc tac atc gcc tcg gac aac acg Glu Ile Ser Ser Gly Asp Glu Phe Tyr Ile Ala Ser Asp Asn Thr 2305 2310 2315			6994
ggg aca ccg ctg gct gtt ttc agc agc aac ggg ctc atg ctg aaa Gly Thr Pro Leu Ala Val Phe Ser Ser Asn Gly Leu Met Leu Lys 2320 2325 2330			7039
cag acc cag tac act gcc tat ggt gag atc tac ttt gac tcc aac Gln Thr Gln Tyr Thr Ala Tyr Gly Glu Ile Tyr Phe Asp Ser Asn 2335 2340 2345			7084
gtc gac ttt cag ctg gta att gga ttc cac ggg ggc ttg tat gac Val Asp Phe Gln Leu Val Ile Gly Phe His Gly Gly Leu Tyr Asp 2350 2355 2360			7129
ccg ctc acc aaa cta atc cac ttt gga gaa aga gat tat gac att Pro Leu Thr Lys Leu Ile His Phe Gly Glu Arg Asp Tyr Asp Ile 2365 2370 2375			7174
ttg gcg gga aga tgg acc aca ccg gac att gaa atc tgg aaa agg Leu Ala Gly Arg Trp Thr Thr Pro Asp Ile Glu Ile Trp Lys Arg 2380 2385 2390			7219
atc gga aag gac cct gct cct ttt aac ctg tat atg ttt cgg aat			7264

80/88

Ile Gly Lys Asp Pro	Ala Pro Phe Asn Leu Tyr Met Phe Arg Asn	2395	2400	2405	
aac aac ccc gcg agc	aaa atc cat gat gtg	aaa gat tac atc acg			7309
Asn Asn Pro Ala Ser	Lys Ile His Asp Val	Lys Asp Tyr Ile Thr	2410	2415	2420
gat gtt aac agc tgg	ctg gtg acg ttt ggc	ttc cat ctg cac aat			7354
Asp Val Asn Ser Trp	Leu Val Thr Phe Gly	Phe His Leu His Asn	2425	2430	2435
gct att cct gga ttc	cct gtt ccc aaa ttt	gat tta act gag cct			7399
Ala Ile Pro Gly Phe	Pro Val Pro Lys Phe	Asp Leu Thr Glu Pro	2440	2445	2450
tcc tat gag ctt gtg	aag agt caa cag tgg	gaa gat gtg ccg ccc			7444
Ser Tyr Glu Leu Val	Lys Ser Gln Gln Trp	Glu Asp Val Pro Pro	2455	2460	2465
atc ttt gga gtt cag	cag caa gtg gca agg	caa gcc aag gcc ttc			7489
Ile Phe Gly Val Gln	Gln Gln Val Ala Arg	Gln Ala Lys Ala Phe	2470	2475	2480
ttg tcc ctg ggg aag	atg gcc gag gtg cag	gtg agc cga cgc aaa			7534
Leu Ser Leu Gly Lys	Met Ala Glu Val Gln	Val Ser Arg Arg Lys	2485	2490	2495
gct ggc gcc gag cag	tcg tgg ctg tgg ttc	gcc acg gtc aag tcg			7579
Ala Gly Ala Glu Gln	Ser Trp Leu Trp Phe	Ala Thr Val Lys Ser	2500	2505	2510
ctc atc ggc aag ggc	gtc atg ctg gcc gtg	agc caa ggc cgc gtg			7624
Leu Ile Gly Lys Gly	Val Met Leu Ala Val	Ser Gln Gly Arg Val	2515	2520	2525
cag acc aac gtg ctc	aac atc gcc aac gag	gac tgc atc aag gtg			7669
Gln Thr Asn Val Leu	Asn Ile Ala Asn Glu	Asp Cys Ile Lys Val	2530	2535	2540
gcg gcg gtg ctc aac	aac gcc ttc tac ctg	gag aac ctg cac ttc			7714
Ala Ala Val Leu Asn	Asn Ala Phe Tyr Leu	Glu Asn Leu His Phe	2545	2550	2555
acc atc gag ggc aag	gac aca cac tac ttc	atc aag acc acc aca			7759
Thr Ile Glu Gly Lys	Asp Thr His Tyr Phe	Ile Lys Thr Thr Thr	2560	2565	2570
ccc gag agc gac ctg	ggc aca ctg cgg ctg	acg agc ggt cgc aag			7804
Pro Glu Ser Asp Leu	Gly Thr Leu Arg Leu	Thr Ser Gly Arg Lys	2575	2580	2585
gcc ctg gag aac ggg	atc aac gtg acc gtg	tct cag tcc acc acg			7849
Ala Leu Glu Asn Gly	Ile Asn Val Thr Val	Ser Gln Ser Thr Thr	2590	2595	2600
gtg gtg aac ggc agg	act cgc agg ttc gcc	gac gtg gag atg cag			7894
Val Val Asn Gly Arg	Thr Arg Arg Phe Ala	Asp Val Glu Met Gln	2605	2610	2615

81/88

ttc ggt gcc ctg gca ctg cat gtg cgc tat ggc atg acg ctg gac	7939
Phe Gly Ala Leu Ala Leu His Val Arg Tyr Gly Met Thr Leu Asp	
2620 2625 2630	
gag gag aag gcg cgc att ctg gag cag gcg cgc cag cgc gcg ctc	7984
Glu Glu Lys Ala Arg Ile Leu Glu Gln Ala Arg Gln Arg Ala Leu	
2635 2640 2645	
gcc cgg gcg tgg gca cgg gag cag cag cgc gtg cgc gac ggc gag	8029
Ala Arg Ala Trp Ala Arg Glu Gln Gln Arg Val Arg Asp Gly Glu	
2650 2655 2660	
gag ggt gcg cgc ctc tgg acg gag ggt gag aaa cgg cag ctg ctg	8074
Glu Gly Ala Arg Leu Trp Thr Glu Gly Glu Lys Arg Gln Leu Leu	
2665 2670 2675	
agc gct ggc aag gtg cag ggc tac gat ggg tac tac gta ctg tcg	8119
Ser Ala Gly Lys Val Gln Gly Tyr Asp Gly Tyr Tyr Val Leu Ser	
2680 2685 2690	
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Val Glu Gln Tyr Pro Glu Leu Ala Asp Ser Ala Asn Asn Ile Gln	
2695 2700 2705	
ttc ttg cga caa agt gag atc ggc aag agg taa cccccgggcc	8207
Phe Leu Arg Gln Ser Glu Ile Gly Lys Arg	
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accctgtgc agattctcct gtagcacaat ccaaaccgga ctctccaaag agccttccaa	8267
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accctgattt ttttgtaaat tatgtgagac aagttgttta tggattttta tatgaattac	8867
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82/88

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                                     Met Tyr Ser
                                     1

gtt gac cgt gtg tct gac gac atc cct att cgt acc tgg ttc ccc aag      163
Val Asp Arg Val Ser Asp Asp Ile Pro Ile Arg Thr Trp Phe Pro Lys
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gaa aat ctt ttc agc ttc cag aca gca acc aca act atg caa gcg gtg      211
Glu Asn Leu Phe Ser Phe Gln Thr Ala Thr Thr Thr Met Gln Ala Val
20                               25                               30                               35

ttc agg ggc tac gcg gag agg aag cgc cgg aaa cgg gag aat gat tcc      259
Phe Arg Gly Tyr Ala Glu Arg Lys Arg Arg Lys Arg Glu Asn Asp Ser
                               40                               45                               50

gcg tct gta atc cag agg aac ttc cgc aaa cac ctg cgc atg gtc ggc      307
Ala Ser Val Ile Gln Arg Asn Phe Arg Lys His Leu Arg Met Val Gly
                               55                               60                               65

agc cgg agg gtg aag gcc cag acg ttc gct gag cgg cgc gag cgg agc      355
Ser Arg Arg Val Lys Ala Gln Thr Phe Ala Glu Arg Arg Glu Arg Ser
                               70                               75                               80

ttc agc cgg tcc tgg agc gac ccc acc ccc atg aaa gcc gac act tcc      403
Phe Ser Arg Ser Trp Ser Asp Pro Thr Pro Met Lys Ala Asp Thr Ser
      85                               90                               95

cac gac tcc cga gac agc agt gac ctg cag agc tcc cac tgc acg ctg      451
His Asp Ser Arg Asp Ser Ser Asp Leu Gln Ser Ser His Cys Thr Leu
100                               105                               110                               115

gac gag gcc ttc gag gac ctg gac tgg gac act gag aag gcc ctg gag      499
Asp Glu Ala Phe Glu Asp Leu Asp Trp Asp Thr Glu Lys Gly Leu Glu
                               120                               125                               130

gct gtg gcc tgc gac acc gaa ggc ttc gtg cca cca aag gtc atg ctc      547
Ala Val Ala Cys Asp Thr Glu Gly Phe Val Pro Pro Lys Val Met Leu
                               135                               140                               145

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83/88

att tcc tcc aag gtg ccc aag gct gag tac atc ccc act atc atc cgc	595
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150 155 160	
cgg gat gac ccc tcc atc atc ccc atc ctc tac gac cat gag cac gca	643
Arg Asp Asp Pro Ser Ile Ile Pro Ile Leu Tyr Asp His Glu His Ala	
165 170 175	
acc ttc gag gac atc ctt gag gag ata gag agg aag ctg aac gtc tac	691
Thr Phe Glu Asp Ile Leu Glu Glu Ile Glu Arg Lys Leu Asn Val Tyr	
180 185 190 195	
cac aag gga gcc aag atc tgg aaa atg ctg att ttc tgc cag gga ggt	739
His Lys Gly Ala Lys Ile Trp Lys Met Leu Ile Phe Cys Gln Gly Gly	
200 205 210	
cct gga cac ctc tat ctc ctc aag aac aag gtg gcc acc ttt gcc aaa	787
Pro Gly His Leu Tyr Leu Leu Lys Asn Lys Val Ala Thr Phe Ala Lys	
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gtg gag aag gaa gag gac atg att cac ttc tgg aag cgg ctg agc cgc	835
Val Glu Lys Glu Glu Asp Met Ile His Phe Trp Lys Arg Leu Ser Arg	
230 235 240	
ctg atg agc aaa gtg aac cca gag ccg aac gtc atc cac atc atg ggc	883
Leu Met Ser Lys Val Asn Pro Glu Pro Asn Val Ile His Ile Met Gly	
245 250 255	
tgc tac att ctg ggg aac ccc aat gga gag aag ctg ttc cag aac ctc	931
Cys Tyr Ile Leu Gly Asn Pro Asn Gly Glu Lys Leu Phe Gln Asn Leu	
260 265 270 275	
agg acc ctc atg act cct tat agg gtc acc ttc gag tca ccc ctg gag	979
Arg Thr Leu Met Thr Pro Tyr Arg Val Thr Phe Glu Ser Pro Leu Glu	
280 285 290	
ctc tca gcc caa ggg aag cag atg atc gag acg tac ttt gac ttc cgg	1027
Leu Ser Ala Gln Gly Lys Gln Met Ile Glu Thr Tyr Phe Asp Phe Arg	
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Leu Tyr Arg Leu Trp Lys Ser Arg Gln His Ser Lys Leu Leu Asp Phe	
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Asp Asp Val Leu	
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84/88

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<212> DNA

<213> Danio rerio

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<400> 134

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tgg gct gga cca gcc acc tct gcc tga gac ctc cgg tcg ccg caa gaa 96
 Trp Ala Gly Pro Ala Thr Ser Ala Asp Leu Arg Ser Pro Gln Glu
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85/88

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Ser Tyr Leu Val Pro Gln Gly Lys Ser Phe Gln Leu Pro Asp Ser Asn			
50	55	60	
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His Asn Tyr Ala Ser Gly Val Gln Gly Leu Arg Gly Glu Glu Ala Pro			
65	70	75	
gaa acg gga gaa tga ttc cgc gtc tgt aat cca gag gaa ctt ccg caa			288
Glu Thr Gly Glu Phe Arg Val Cys Asn Pro Glu Glu Leu Pro Gln			
80	85	90	
aca cct gcg c atggtcggca gccggagggt gaaggcccag acgttcgctg			338
Thr Pro Ala			
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ggaagcggct gagccgcctg atgagcaaag tgaaccaga gccgaacgtc atccacatca			878
tgggctgcta cattctgggg aacccaatg gagagaagct gttccagaac ctcaggacc			938
tcatgactcc ttatagggtc accttcgagt caccctgga gctctcagcc caaggggaagc			998
agatgatcga gacgtacttt gacttcoggt tgtatcgcct gtggaagagc cgccagcact			1058
cgaagctgct ggactttgac gacgtcctgt gaggggcaga ggctccgcc cagtcaccat			1118
caggccactc cctctgcacc gggacctggg gctgggcccgc ctcgtgctcc ccgggactgt			1178
gtagctccgg tctcgcctgg agccacttca gggcacctca gacgttgctc aggttcccc			1238
tgtgggttcc ggtcctcgtc gcacctggt cgcagaggc tgcagtcctt gggggccggg			1298
aggatcccgc cctgtggccc gtggatgctc agcggccagg cactgacctg ccatgcctcg			1358
cctggaggct cagctgtggg catccctcca tggggttcat agaaataagt gcaatttcta			1418
cacccccgaa acaattcaaa gggaagcagc atttcttgtt aactagttaa gcactatgct			1478
gctagttaca gtgtaggcac ccgggccag cagcccagca gccacatgt gttcaggacc			1538
ctccctgccc acccctccc tgccgtatcg atcaccagca ccaggggtggc ccgtgtgcgt			1598

86/88

ggggccagcg tcgccgggct gccagcctg gctctgtcta cactggccga gtctctgggt 1658
 ctgtctacac tggccgagtc tccgactgtc tgtgctttca cttacactcc tcttgccacc 1718
 ccccatccct gcttacttag acctcagccg gcgccggacc cggtaggggc agtctgggca 1778
 gcaggaagga agggcgcagc gtcccctcct tcagaggagg ctctgggtgg ggcctgctcc 1838
 tcatcccccc aagcccaccc agcactctca ttgctgctgt tgagttcagc ttttaccagc 1898
 ctcagtgtgg aggctccatc ccagcacaca ggcctggggc ttggcagggg cccagctggg 1958
 gctgggccct gggttttgag aaactcgtcgc gcaccacagt gggcccctgg acccggccgc 2018
 gcagctgggtg gactgtaggg gctcctgact gggcacagga gctcccagct tttgtccacg 2078
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<212> PRT

<213> Artificial Sequence

<220>

<223> TCAP 3 General Motif

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<222> (5)..(5)

<223> X=G, S or A

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> X=G or R

<220>

87/88

<221> MISC_FEATURE

<222> (9) .. (9)

<223> X=L or Q

<220>

<221> MISC_FEATURE

<222> (19) .. (19)

<223> X=V or I

<220>

<221> MISC_FEATURE

<222> (32) .. (32)

<223> X=V or I

<400> 135

Gln	Leu	Leu	Ser	Xaa	Xaa	Lys	Val	Xaa	Gly	Tyr	Asp	Gly	Tyr	Tyr	Val
1				5					10					15	

Leu	Ser	Xaa	Glu	Gln	Tyr	Pro	Glu	Leu	Ala	Asp	Ser	Ala	Asn	Asn	Xaa
			20					25					30		

Gln	Phe	Leu	Arg	Gln	Ser	Glu	Ile
		35					40

<210> 136

<211> 36

<212> PRT

<213> Artificial Sequence

<220>

<223> G. gallus TCAP2

<400> 136

88/88

Thr Gly Arg Val Gln Gly Tyr Glu Gly Tyr Tyr Val Leu Pro Val Glu
1 5 10 15

Gln Tyr Pro Glu Leu Ala Asp Ser Ser Ser Asn Ile Gln Phe Leu Arg
 20 25 30

Gln Asn Glu Met
 35

-95 -

WE CLAIM:

1. An isolated nucleic acid molecule encoding a teneurin c-terminal associated peptide consisting of:
 - 5 (a) a nucleic acid sequence as shown in SEQ.ID.NOS.: 18-20, 25-28, 33-36, 41-44, 49-52, 57-60, 65-68, 73-76, 81-84, 89-92, 97-100 or that wherein T can also be U or that encodes a peptide having an amino acid sequence selected from the group consisting of : SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or
10 that further has an amidation signal sequence, at the carboxy terminus of said peptides, or has SEQ. ID. NO. 15, 16, 23, 24, 31, 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96;
 - 15 (b) a nucleic acid sequence that is complimentary to a nucleic acid sequence of (a);
 - (c) a nucleic acid sequence that has substantial sequence homology to a nucleic acid sequence of (a) or (b);
 - (d) a nucleic acid sequence that is an analog of a nucleic acid sequence of (a), (b) or (c); or
 - 20 (e) a nucleic acid sequence that hybridizes to a nucleic acid sequence of (a), (b), (c) or (d) under stringent hybridization conditions.
2. A isolated nucleic acid molecule of claim 1 wherein the amidation signal
25 sequence is GKR or GRR.
3. A nucleic acid molecule of claim 2 wherein the sequence is selected from the group of sequences consisting of SEQ. ID. NOS:15, 16, 23, 24, 31, 32, 39, 40, 47, 48, 55, 56, 63, 64, 71, 72, 79, 80, 97, 88, 95, 96.

30

-96 -

4. An isolated nucleic acid molecule encoding a TCAP peptide wherein the peptide has neuronal communication activity and/or stress modulation activity and/or cell proliferation inhibition activity.
- 5 5. An antisense oligonucleotide that is complimentary to a nucleic acid sequence according to claims 1 to 4.
6. An expression vector comprising a nucleic acid molecule of any one of claims 1 to 5.
- 10 7. A host cell transformed with an expression vector of claim 6.
8. An isolated teneurin c-terminal associated peptide which has the amino acid sequence as shown in SEQ. ID. NOS: 13, 14, 21, 22, 29, 30, 37, 15 38, 45, 46, 53, 54, 61, 62, 69, 70, 77, 78, 85, 86, 93, 94, 101, 103 or a fragment, analog, homolog, derivative or mimetic thereof or a biologically active fragment thereof.
9. An isolated teneurin c-terminal associated peptide of claim 8 further 20 comprising an amidation signal sequence at the carboxy terminus.
10. A teneurin c-terminal associated peptide according to claim 8 or 9 wherein the peptide has angiogenic activity.
- 25 11. An antibody that can bind a peptide according to any one of claims 8 to 10 .
12. A method of identifying substances which can bind with a teneurin c-terminal associated peptide, comprising the steps of:
30 (a) incubating a teneurin c-terminal associated peptide and a test substance, under conditions which allow for formation of a

-97 -

complex between the teneurin c-terminal associated peptide and the test substance, and

- 5 (b) assaying for complexes of the teneurin c-terminal associated peptide and the test substance, for free substance or for non complexed teneurin c-terminal associated peptide, wherein the presence of complexes or reduced levels as compared to a starting level of free substance or non-complexed teneurin c-terminal associated peptide indicates that the test substance is capable of binding to the teneurin c-terminal associated peptide.

10

13. A method for identifying a compound that affects the activity or expression of teneurin c-terminal associated peptide comprising:

- 15 (a) incubating a test compound with a teneurin c-terminal associated peptide or a nucleic acid encoding a teneurin c-terminal associated peptide; and

- (b) determining an amount of teneurin c-terminal associated peptide protein activity or expression and comparing with a control, wherein a change in the TCAP peptide activity or expression as compared to the control indicates that the test compound has an effect on TCAP peptide activity or expression.

20

14. The method of claim 13 wherein in step(a) a test compound is incubated with a teneurin c-terminal associated peptide and teneurin c-terminal associated peptide substrate under conditions that permit interaction of the peptide and substrate, and step(b) and in step(b) the peptide activity on the substrate is determined.

25

15. The method of claim 13, wherein in step (a) a cell expressing a teneurin c-terminal associated peptide and activity, is incubated with a test compound, under conditions where teneurin c-terminal associated peptide is active and in step (b) teneurin c-terminal associated peptide activity is determined.

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-98 -

16. The method of claim 15, wherein the teneurin c-terminal associated peptide activity is determined by detecting the levels of cAMP and cGMP before and after incubation with the test compound, or as compared to a control, wherein a change in magnitude of levels of cAMP or cGMP as compared to a baseline or control level is indicative that the test compound is a modulator of teneurin c-terminal associated peptide activity.
17. The method of claim 16, wherein the reduction of cAMP or cGMP in the presence of a test compound is less than in the control or baseline level or is greater than in the control or baseline level of TCAP activity indicates that the test compound is an inhibitor of c-teneurin associated peptide activity.
18. A method of identifying a compound that affects the regulation of neuronal growth comprising:
- (a) incubating a test compound with a teneurin c-terminal associated peptide or a nucleic acid encoding a teneurin c-terminal associated peptide; and
 - (b) determining an amount of teneurin c-terminal associated peptide protein activity or expression and comparing with a control, wherein a change in the TCAP peptide activity or expression as compared to the control indicates that the test compound has an effect on the regulation of neuronal growth.
19. A method of inhibiting cell proliferation comprising administering to a cell, an effective amount of teneurin c-terminal associated peptide that inhibits cell proliferation.
20. A method according to claim 19 wherein the cell is selected from the group consisting of neuronal or fibroblast cells.

-99 -

21. A method of detecting a condition associated with the aberrant regulation of neuronal growth comprising assaying a sample for (a) a nucleic acid molecule encoding a teneurin c-terminal associated peptide or a fragment thereof or (b) a teneurin c-terminal associated peptide or a fragment thereof.
22. A method of treating a condition associated with the aberrant regulation of neuronal growth comprising administering to a cell or animal in need thereof, an effective amount of a teneurin c-terminal associated peptide or an agent that modulates teneurin c-terminal associated peptide expression and/or activity.
23. A method according to claim 22 wherein the agent is selected from the group consisting of: a nucleic acid molecule encoding teneurin c-terminal associated peptide; teneurin c-terminal associated peptide as well as fragments, analogs, derivatives or homologs thereof; antibodies; antisense nucleic acids; peptide mimetics; and substances isolated using the screening methods described in claims 12- 20.
24. A method of inducing an angiogenic response in a subject comprising administering to a subject an effective amount of teneurin c-terminal associated peptide to induce an angiogenic response.
25. A method of inhibiting an angiogenic response in a subject comprising administering to a subject an effective amount of an inhibitor of teneurin c-terminal associated peptide to inhibit an angiogenic response.
26. A method of claim 25 wherein the inhibitor is identified according to the method of anyone of claims 13 to 18.

-100 -

27. A method of inhibiting the damage caused by physiological stresses comprising administering to a cell, an effective amount of teneurin c-terminal associated peptide that protects cells from the physiological stresses.
- 5
28. A method of modulating the stress response in an animal comprising administering an effective amount of TCAP to said animal.
29. A method of modulating anxiety response in an animal comprising administering an effective amount of TCAP to said animal.
- 10
30. The method of increasing anxiety in a low anxiety animal comprising administering to said animal an effective amount of TCAP.
- 15 31. A method of decreasing anxiety in a high anxiety animal comprising administering to said animal an effective amount of TCAP.
32. A method of normalizing anxiety response in an animal comprising administering to said animal an effective amount of TCAP.
- 20
33. A method of treating cancer in an animal comprising administering an effective amount of TCAP to said animal.
34. A pharmaceutical composition comprising TCAP and a pharmaceutically acceptable vehicle.
- 25

1/30

FIGURE 1

5'-tccatctcgggggtgcaacaggaagtgacccggcaagccaaggctttcctgtccttcgag 60
 S I S G V Q Q E V T R Q A K A F L S F E 20
 aggatgccggagatccagctgagccgcccggcgctccaaccgggagaaaccctggctgtgg 120
 R M P E I Q L S R R R S N R E K P W L W 40
 ttcgccaccgccaagtctctgatcggtaaggggtgtcatgttgccgggtgacgcagggcgt 180
 F A T A K S L I G K G V M L A V T Q G R 60
 gtggtcaccaacgctctgaacatcgccaacgaggactgcatcaaggctcgccgcccgtcctc 240
 V V T N A L N I A N E D C I K V A A V L 80
 aacaatgcgcttctacctggaggacctgcacttcacgggtggaggggacgcgacacgcactac 300
 N N A F Y L E D L H F T V E G R D T H Y 100
 ttcataagaccagcctcccggagagcgacctgggagcgctgaggctgacaagcgggagg 360
 F I K T S L P E S D L G A L R L T S G R 120
 aagtcgctggagaacggaagtcaacgtgactgtgtcccagtcaccaccgtggtgaacgg 420
 K S L E N G V N V T V S Q S T T V V N G 140
 cagaaccggcgcttcgcccagctggagctgcagtacggcgctctagcgctccacgtgcgc 480
 R T R R F A D V E L Q Y G A L A L H V R 160
 tatggcatgactctggacgaggagaaggcgctgtgctggagcaggccaggcagaaggcg 540
 Y G M T L D E E K A R V L E Q A R Q K A 180
 ttgtcgagtgcctgggtccagggagcaacaacgggtgagggagggggaggaggggggtgagg 600
 L S S A W S R E Q Q R V R E G E E G V R 200
 ctgtggacggagggggagaagaggcagctgctgagcgggaggaaggttctgggctacgac 660
 L W T E G E K R Q L L S G R K V L G Y D 220
 gggactacgtcctctccatagagcagtagccccgagctagcagactccgctaacaacatc 720
 G Y Y V L S I E Q Y P E L A D S A N N I 240
 cagttcctcaggcagagcgaatataggggaagaggtaa (SEQ.ID.NO.2) 756
 Q F L R Q S E I G K R stop (SEQ.ID.NO.3) 251
 cagacagaatcctcggcactggcc 780
 gccaaagagactaccccctccaaatcctgcccccaacctccctcgcctcccccttttc 840
 tctaaaaagggggaggggtccaggctagtgtgtgttttagcgcgactagctgaaacaaac 900
 agtaaaatgtagaatatcttaaactgaactatacctaataactaccactgtggggcctgaa 960
 aatcaaaacaaaacggctccaactgacgcaaagtgtttgtcccatgtgtctatacagcgttga 1020
 atggactgtggactctcttgaaaagagagaaaaaaaagtcaaaactctcggttttgtgaaa 1080
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 taatagaagaaaagccttctggttttcttacacaggacaacgtctataatctgattctaca 1260
 tcctgacgactgacctttgattgacctttgcgtactgaaaaaggtagtgtttgtttcgc 1320
 agtaggaccatgggtctccaatgggtggttaactagacagttaaaaccacttgttgaaacca 1380
 cttgcttgttcttctgctttttctttccaaaagggacaaaacagctcccaccaagtgactt 1440
 ctttaccataactagatcaaagtgggacgtttttgggctcgtgccgaattc-3' (SEQ.ID.NO.1) 1490

2/30

FIGURE 2

O.mykiss Ten M3	SISGVQQEVTRQAKAFLSFERMPEIQLSRRRSNREKPWLWFATAKSLIGK
R.danio Ten M3	SISGVQQEVMRQAKAFLSFERMPEIQLSRRRSSREKPWLWFATVKSLIGK
M.musculus Ten M3	PIFGVQQOVARQAKAFLSLGKMAEVQVSRKAGAEQSWLWFATVKSLIGK
H.sapiens Ten M3	PIFGVQQOVARQAKAFLSLGKMAEVQVSRRRAGGAQSWLWFATVKSLIGK
O.mykiss Ten M3	GVMLAVT QGRVVTNALNIANEDCIKVAAVLNNAFYLEDLHFTVEGRDTH
R.danio Ten M3	GVMLAITSKGQVATNALNIANEDCIKVVTVLNNAFYLEDLHFTVEGRDTH
M.musculus Ten M3	GVMLAVS QGRVQTNVLNIANEDCIKVAAVLNNAFYLENLHFTIEGKDTH
H.sapiens Ten M4	GVMLAVS QGRVQTNVLNIANEDCIKVAAVLNNAFYLENLHFTIEGKDTH
O.mykiss Ten M3	YFIKTSLPESDLGALRLTSGRKSLENGVNVTVSQSTTVVNGRTRRFADVE
R.danio Ten M3	YFIKTSLPESDLGALRLTSGRKSLENGVNVTVSQSTTVVNGRTRRFADVE
M.musculus Ten M3	YFIKTTTPESDLGTLRLTSGRKALENGINVTVSQSTTVVNGRTRRFADVE
H.sapiens Ten M3	YFIKTTTPESDLGTLRLTSGRKALENGINVTVSQSTTVVNGRTRRFADVE
O.mykiss Ten M3	LOYGALALHVRYGMTLDEEKARVLEQARQALSSAWSREQQRVREGGEGV
R.danio Ten M3	LOYGALALHVRYGMTLDEEKARVLEQARQALSSAWAREQQRVRDGGEGV
M. musculus Ten M3	MOFGALALHVRYGMTLDEEKARILEQARQALARAWAREQQRVRDGGEEGA
H. sapiens Ten M3	MOFGALALHVRYGMTLDEEKARILEQARQALARAWAREQQRVRDGGEEGA
O.mykiss Ten M3	RLWTEGEKRQLLSGRKVLGYDGYVLSIEQYPELADSANNIQFLRQSEIG
R.danio Ten M3	RLWTEGEKRQLLSGKVLGYDGYVLSVEQYPELADSANNVQFLRQSEIG
M.musculus Ten M3	RLWTEGEKRQLLSAGKVQGYDGYVLSVEQYPELADSANNIQFLRQSEIG
H. sapiens M3	RLWTEGEKRQLLSAGKVQGYDGYVLSVEQYPELADSANNIQFLRQSEIG
O. mykiss Ten M3	KR (SEQ.ID.NO.3)
R. danio Ten M3	KR (SEQ.ID.NO.12)
M. musculus Ten M3	KR (SEQ.ID.NO.6)
H. sapiens Ten M3	RR (SEQ.ID.NO.10)

3/30

FIGURE 3

Mouse Teneurin 1	MILGIQCELOKQLRNFI SLDQLPMTPOYNEGRCLGEGKQPRFAAVPSVFG
Mouse Ten eurin M2	LITGVQQTTERHNQAFLEAGQVITKKLHAS IREKAGHWFATTTPIIG
Mouse Ten eurin M3	PIFGVQQQVARQAKAFLSL GKMAEVQVSRKAGAEQSWLWFATVKSLIG
Mouse Ten eurin M4	SILGVQCEVQKQLKAFVTLERFDQLYGSTITSCQQAPETKKFASSGSIFG
Mouse Teneurin 1	KGIKFAIKEGIVTADIIGVANEDSRRLAAILNNAHYLENLHFTIEGRDTH
Mouse Teneurin 2	KGIMFAIKEGRVTTGVSSIASEDSRKVASVLNNAYYLDKMHSIEGKDTH
Mouse Teneurin 3	KGVMLAVSQGRVQTNVLNIANEDCIKVA AVLNNAFYLENLHFTIEGKDTH
Mouse Teneurin 4	KGVKFALKDGRVTTDII SVANEDGRRIAAILNNAHYLENLHFTIDGVDTH
Mouse Teneurin 1	YFIKLGSL EEDLV LIGNTGRRILENGVNVTVSQM TSVLNGRTRRFADIQ
Mouse Teneurin 2	YFVKIGAADGDLVTLGTTIGRKVLESGVNVTVSQPTLLVNGRTRRFNIE
Mouse Teneurin 3	YFIKTTTPESDLGTLRLTSGRKALENGINVTVSQSTTVVNGRTRRFADVE
Mouse Teneurin 4	YFVKPGPSEGDLAILGLSGRR TLENGVNVTVSQINTML
Mouse Teneurin 1	LQHGALCFNIRYGT VEEKKNHVLEMARQRAVAQAWTQEQRRLQEGE
Mouse Teneurin 2	FQYSTLLLSIRYGLTPDTLDEEKARVLDQAGQRALGTAWAKEQQKARDGR
Mouse Teneurin 3	MQFGALALHVRYGMT LDEEKARILEQARQRALARAWAREQQRVRDGE
Mouse Teneurin 4	IQLQYRALCLNTRYGT TVDEEKVRVLELARQRAVRQAWAREQQRLREGE
Mouse Teneurin 1	EGTRVWTEGEKQQLLGTGRVQGYDGYFVLSVEQYLELSDSANNIHFMRS
Mouse Teneurin 2	EGSRLWTEGEKQQLLSTGRVQGYEGYYVLPVEQYPELADSSNIQFLRQN
Mouse Teneurin 3	EGARLWTEGEKRQLLSAGKVQGYDGYVLSVEQYPELADSANNIQFLRQS
Mouse Teneurin 4	EGLRAWTDGEKQQLNTGRVQGYDGFVTSVEQYPELSDSANNIHFMRS
Mouse Teneurin 1	EIGRR (SEQ. ID. NO. 4)
Mouse Teneurin 2	EMGKR (SEQ. ID. NO. 5)
Mouse Teneurin 3	EIGKR (SEQ. ID. NO. 6)
Mouse Teneurin 4	EMGRR (SEQ. ID. NO. 7)

4/30

FIGURE 4

Human Ten M1	TILGIQCELOKQLRNFISL	D QLPMTPRYNDGRCLEGGKQ	PRFA
Human Ten M2	LITGVQQTTERHNQAFMALE	GQV ITKKLHASIREKAGHW	FA
Human Ten M3	PIFGVQQQVARQAKAFSLGKMAEVQV	SRRRAGGA	QS WLW FA
Human Ten M4	SILGVQCEVQKQLKAFVTLER	FD QL YGSTITSCLQAPKT	KKFA
Human Ten M1	AVPSVFGKGIKFAIKDGIVTADIIGVANEDSRRLAAILNNAHYLENLHFT		
Human Ten M2	TTTPIIGKGMFAIKEGRVTTGVSSIASEDSRKVASVLNNAAYLDKMHYS		
Human Ten M3	TVKSLIGKGVMLAVSQGRVQTNVLNIANEDCIKVAAVLNNAFYLENLHFT		
Human Ten M4	SSGSVFGKGVKFAIKDGRVTTDII SVANEDGRRVAAILNHAHYLENLHFT		
Human Ten M1	IEGRDTHYFIKLGSLLEEDLVLIGNTGGRRILENGVNVTVSQMTSVLNGRT		
Human Ten M2	IEGKDTHYFVKIGSADGDLVTLGTTIGRKVLESGVNVTVSQPTLLVNGRT		
Human Ten M3	IEGKDTHYFIKTTTPESDLGTLRLTSGRKALENGINVTVSQSTTVVNGRT		
Human Ten M4	IDGVDTHYFVKPGPSEGLAILGLSGGRRTLENGVNVTVSQINTVLSGRT		
Human Ten M1	RRFADIQLQHGALCFNIRYGT	VEEEKNHVLEIARQRAVAQAWTKEQ	
Human Ten M2	RRFTNIEFQYSTLLLSIRYGLTPDTLDEEKARVLDQARQALGTAWAKEQ		
Human Ten M3	RRFADVEMQFGALALHVRYGMT	LDEEKARILEQARQALARAWAREQ	
Human Ten M4	RRYTDIQLQYGALCLNTRYGT	LDEEKARVLELARQRAVRQAWAREQ	
Human Ten M1	RRLQEGEEGIRAWTEGEKQQLLSTGRVQGYDGYFVLSVEQYLELSDSANN		
Human Ten M2	QKARDGREGSRLWTEGEKQQLLSTGRVQGYEGYYVLPVEQYPELADSSSN		
Human Ten M3	QRVRDGEEGARLWTEGEKQQLSAGKVQGYDGYVLSVEQYPELADSSANN		
Human Ten M4	QRLREGEEGLRAWTEGEKQQVLSTGRVQGYDGGFFVISVEQYPELSDSANN		
Human Ten M1	IHFMRQSEIGRR	(SEQ. ID. NO. 8)	
Human Ten M2	IQFLRQNE MGKR	(SEQ. ID. NO. 9)	
Human Ten M3	IQFLRQSEIGRR	(SEQ. ID. NO. 10)	
Human Ten M4	IHFMRQSEMGR	(SEQ. ID. NO. 11)	

5/30

FIGURE 5

Human TCAP-1

cag cag ctt ttg agc act ggg cgg gta caa
 ggt tac gat ggg tat ttt gtt ttg tct gtt
 gag cag tat tta gaa ctt tct gac agt gcc
 aat aat att cac ttt atg aga cag agc gaa
 ata ggc agg agg taa

(SEQ.ID.NO.76
 +stop codon)

Human TCAP-2

cag cag ctt ctg agc acc ggg cgc gtg caa
 ggg tac gag gga tat tac gtg ctt ccc gtg
 gag caa tac cca gag ctt gca gac agt agc
 agc aac atc cag ttt tta aga cag aat gag
 atg gga aag agg taa

(SEQ.ID.NO.84
 +stop codon)

Human TCAP-3

cgg cag ctg ctg agc gcc ggc aag gtg cag
 ggc tac gac ggg tac tac gta ctc tcg gtg
 gag cag tac ccc gag ctg gcc gac agc gcc
 aac aac atc cag ttc ctg cgg cag agc gag
 atc ggc agg agg taa

(SEQ.ID.NO.92
 +stop codon)

Human TCAP-4

cag cag gtg ctg agc aca ggg cgg gtg caa
 ggc tac gac ggc ttt ttc gtg atc tct gtc
 gag cag tac cca gaa ctg tca gac agc gcc
 aac aac atc cac ttc atg aga cag agc gag
 atg ggc cgg agg tga

(SEQ.ID.NO.100
 +stop codon)

Mouse TCAP-1

cag cag ctt ttg ggc acc ggg agg gtg cag
 ggg tat gat ggg tat ttt gtc ttg tct gtt
 gag cag tat tta gaa ctt tca gac agt gcc
 aac aat att cac ttc atg aga cag agt gaa
 ata ggc agg agg taa

(SEQ.ID.NO.44
 +stop codon)

6/30

FIGURE 5 (CONT'D)

Mouse TCAP-2

cag caa ctc ctg agc acg gga cgg gta caa
 ggt tat gag ggc tat tac gta ctt ccg gtg
 gaa cag tac ccg gag ctg gca gac agt agc (SEQ.ID.NO.52
 agc aac atc cag ttc tta aga cag aat gag +stop codon)
 atg gga aag agg taa

Mouse TCAP-3

cgg cag ctg ctg agc gct ggc aag gtg cag
 ggc tac gat ggg tac tac gta ctg tcg gtg
 gag cag tac ccc gag ctg gct gac agt gcc (SEQ.ID.NO.60
 aac aac atc cag ttc ttg cga caa agt gag +stop codon)
 atc ggc aag agg taa

Mouse TCAP-4

cag cag gtg ctg aac acg ggg cgg gtg caa
 ggc tac gac ggc ttc ttt gtg acc tcg gtc
 gag cag tac cca gaa ctg tca gac agc gcc (SEQ.ID.NO.68
 aac aat atc cac ttc atg aga cag agc gag +stop codon)
 atg ggc cga agg tga

Zebrafish TCAP-3

agg cag ttg ctc agc tct ggg aag gtg ctg
 ggt tac gat ggt tac tat gta cta tca gtg
 gag caa tac cct gaa ctg gcc gac agt gcc (SEQ.ID.NO.28
 aac aat gtc cag ttc ttg agg cag agt gag +stop codon)
 ata ggg aag agg taa

Zebrafish TCAP-4

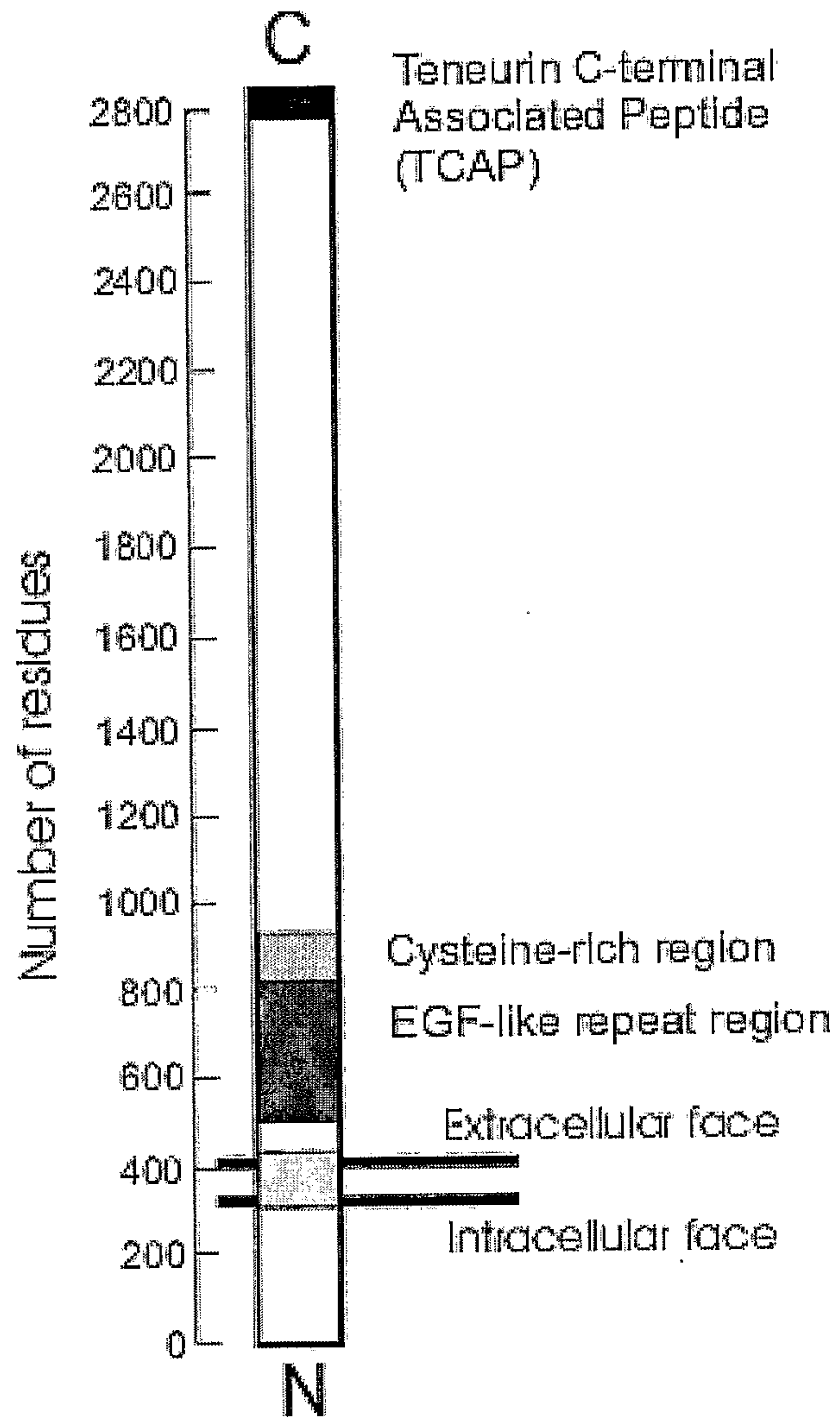
cag cag ctc cta agc tct gga cgt gta cag
 ggc tac gaa ggc ttc tac ata gta tca gtc
 gac cag ttc cca gag ttg act gac aac ata (SEQ.ID.NO.36
 aat aac gtc cat ttc tgg cga cag act gag +stop codon)
 atg gga cgc agg tga

Rainbow Trout TCAP-3

5'-agg cag ctg ctg agc ggg agg aag gtt ctg
 ggc tac gac ggg tac tac gtc ctc tcc ata
 gag cag tac ccc gag cta gca gac tcc gct (SEQ.ID.NO.20
 aac aac atc cag ttc ctc agg cag agc gaa +stop codon)
 ata ggg aag agg taa-3'

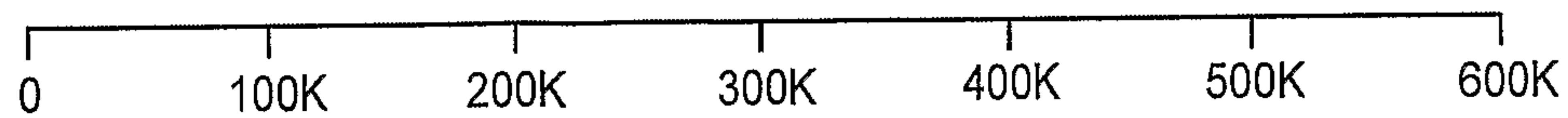
7/30

FIGURE 6A

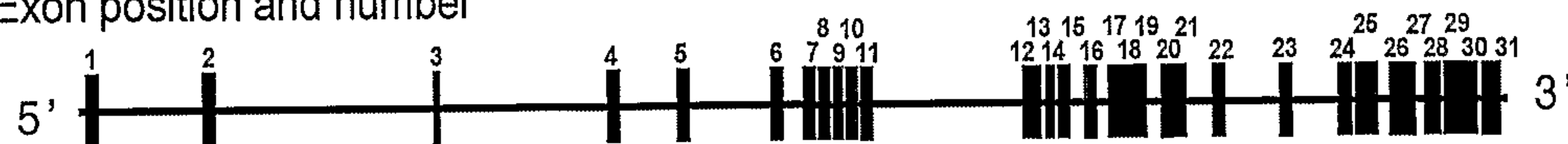


8/30
FIGURE 6B

Number of bases



Exon position and number



Amino acid sequence of C-terminal exon

TILGIQCELOKQLRNFIISLDQLPMTPRYNDGRCLEGGKQPRFAAVPSVFG
 KGIKFAIKDGIIVTAIIGVANEDSRRLAAILNNAHYLENLHFTIEGRDTHY
 FIKLGSLEEDLVLI GNTGGRRILENGVNVTVSQMTSVLNGRTRRFADIQL
 QHGALCFNIRYGTTV EEEKNHVLEIARQRAVAQAWTKEQRRLOEGEEGI R
 AWTEGEKQQLLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI GRR

Accession Numbers

human TCAP 1	QQLLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI-NH2	nm_014253 (SEQ.ID.NO.69)
human TCAP 2	QQLLSTGRVQGYEGYVLPVEQYPELADSSNIQFLRONEM-NH2	xm_047995 (SEQ.ID.NO.78)
human TCAP 3	QQLSAGKVQGYDGYVLSVEQYPELADSSANNIQFLROSEI-NH2	ak001336 (SEQ.ID.NO.85)
human TCAP 4	QQVLSTGRVQGYDGFVLSVEQYPELSDSANNIHFMROSEM-NH2	ak056531 (SEQ.ID.NO.94)
mouse TCAP 1	QQLLGTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI-NH2	nm_011855 (SEQ.ID.NO.37)
mouse TCAP 2	QQLLSTGRVQGYEGYVLPVEQYPELADSSNIQFLRONEM-NH2	nm_011856 (SEQ.ID.NO.76)
mouse TCAP 3	QQLSAGKVQGYDGYVLSVEQYPELADSSANNIQFLROSEI-NH2	nm_011857 (SEQ.ID.NO.53)
mouse TCAP 4	QQVLNTGRVQGYDGFVTSVEQYPELSDSANNIHFMROSEM-NH2	ab025413 (SEQ.ID.NO.66)
Rat TCAP 2	QQLLSTGRVQGYEGYVLPVEQYPELADSSNIQFLRONEM-NH2	nm_020088 (SEQ.ID.NO.78)

9/30

Avian TCAP Sequences

chicken TCAP 1	QQLLNTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI-NH2	aj238613 (SEQ.ID.NO.101)
chicken TCAP 2	QQLLNTGRVQGYEGYVLPVEQYPELADSSNIQFLRONEM-NH2	aj279031 (SEQ.ID.NO.136)

Piscine TCAP Sequences

Rainbow trout TCAP 3	QQLSGRKVLGYDGYVLSIEQYPELADSSANNIQFLROSEI-NH2	not entered	Yet (SEQ.ID.NO.13)
zebrafish TCAP 3	QQLSSGKVLGYDGYVLSVEQYPELADSSANNVQFLROSEI-NH2	nm_130968 (SEQ.ID.NO.21)	
zebrafish TCAP 4	QQLSSGRVQGYEGFYIVSVDQFPPELTDINNVHFWROTEM-NH2	ab026980 (SEQ.ID.NO.30)	
Insect Drosopholia	ELVQHGDVDGWNG1DIHSIHKYPQLADOPGNVAFQRDAK	(SEQ.ID.NO.103)	

FIGURE 7A

10/30

FIGURE 7B

Protein name	Species	Truncated peptide	% Identical	% Homolog
Ten-m1/odd Oz1	<i>M musculus</i>	QLLGTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI	100	
Teneurin-1	<i>G gallus</i>	QLLNTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI	97	97
Odz (odd Oz1/ten-m1) / tenascin M	<i>H sapiens</i>	QLLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI	97	97
Mouse DOC4-like protein	<i>H sapiens</i>	QLLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMROSEI	97	97
DOC4/Ten-m4 /odd Oz4	<i>M musculus</i>	QVLNTGRVQGYDGYFVLSVEQYPELSDSANNIHFMROSEM	85	92
Similar to odd Oz4/ten-m4/ KIAA1302 protein	<i>H sapiens</i>	QVLSTGRVQGYDGYFVLSVEQYPELSDSANNIHFMROSEM	85	95
Hypothetical protein/ DKFZp564O0423.1 (fragment)	<i>H sapiens</i>	QVLSTGRVQGYDGYFVLSVEQYPELSDSANNIHFMROSEM	85	95
odd Oz/ten-m3/ ODZ3	<i>M musculus</i>	QLLSAGKVGQYDGYVLSVEQYPELADSAANNIQFLROSEI	80	90
Hypothetical protein FLJ10474; FLJ10886; unnamed protein products: AK001336, AK027473, AK001748	<i>H sapiens</i>	QLLSAGKVGQYDGYVLSVEQYPELADSAANNIQFLROSEI	80	90
Putative (AK011924)	<i>M musculus</i>	QLLSAGKVGQYDGYVLSVEQYPELADSAANNIQFLROSEI	80	90
N/A	<i>R trout</i>	QLLSGRKVLGYDGYVLSVEQYPELADSAANNIQFLROSEI	80	90
Ten-m3	<i>D rerio</i>	QLLSSGKVLGYDGYVLSVEQYPELADSAANNVQFLROSEI	75	90
Neurestin alpha	<i>R norvegicus</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Teneurin-2	<i>G gallus</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Ten-m2/ ODZ2/ odd Oz2	<i>M musculus</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Odd Oz/ten-m2/ KIAA1127 protein / hypothetical protein	<i>H sapiens</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Hypothetical protein	<i>H sapiens</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Odd Oz/ten-m2	<i>H sapiens</i>	QLLSTGRVQGYEGYVLPVEQYPELADSSSNIQFLRONEM	70	90
Ten-m4	<i>D rerio</i>	QLLSSGRVQGYEGYVMSVQPELADNINNVHFWROTEM	57	89
odd Oz/tenascin-like protein/Ten-m gene product	<i>D melanogaster</i>	ELVQHGVDVGNNGIDTHTSKYPLADDPGNVAFORDAK	30	60

CRF Peptide Family

human CRF	SEPPPSLDLTFHLLREVLLEMARAEQLAQQAHSNRKLMEI I	(SEQ. ID. NO. 104)
human urocortin	DNPSLSLDLTFHLLRTELEARTQSOREAEQNRIFDSV	(SEQ. ID. NO. 105)
human urocortin 2	IVLSLDMPIGLLQIILEQARARAAREQATTNARLARV	(SEQ. ID. NO. 106)
human urocortin 3	FTLSLDVPTNIMNLEFNIAKAKNLRQAANAHLMAQI	(SEQ. ID. NO. 107)

TCAP Peptide Family

human TCAP 1	QQLLSTGRVQGYDGYFVLSVEQYLELSDSANNIHFMRQSEI	(SEQ. ID. NO. 70)
human TCAP 2	QQLLSTGRVQGYEGYVLPVEQYPELADSSNIQFLRQNEM	(SEQ. ID. NO. 78)
human TCAP 3	QLLSAGKVOGYDGYVLSVEQYPELADSSANNIQFLRQSEI	(SEQ. ID. NO. 85)
human TCAP 4	QQVLSLSTGRVQGYDGFVLSVEQYPELSDSANNIHFMRQSEM	(SEQ. ID. NO. 94)

FIGURE 8

	SEQ ID NOS
Human CRF Paralogues	
human CRF	104
human urocortin	105
human urocortin 2	106
human urocortin 3	107
Human TCAP Paralogues	
human TCAP 1	70
human TCAP 2	78
human TCAP 3	85
human TCAP 4	94

SEEPPTSS LDLTFFHLLREVLLEMARAEQLAQQAHSNRKIM EII
 DNPSLS IDLTFHLLRTLELARTQSQRERAQNRI DS
 IVLS LDVPIGLLQIHEQARARAAREQATTNAR AR
 FTLS LDVPTNIMNLFNIAKAKNLRQAANAHLM AQL

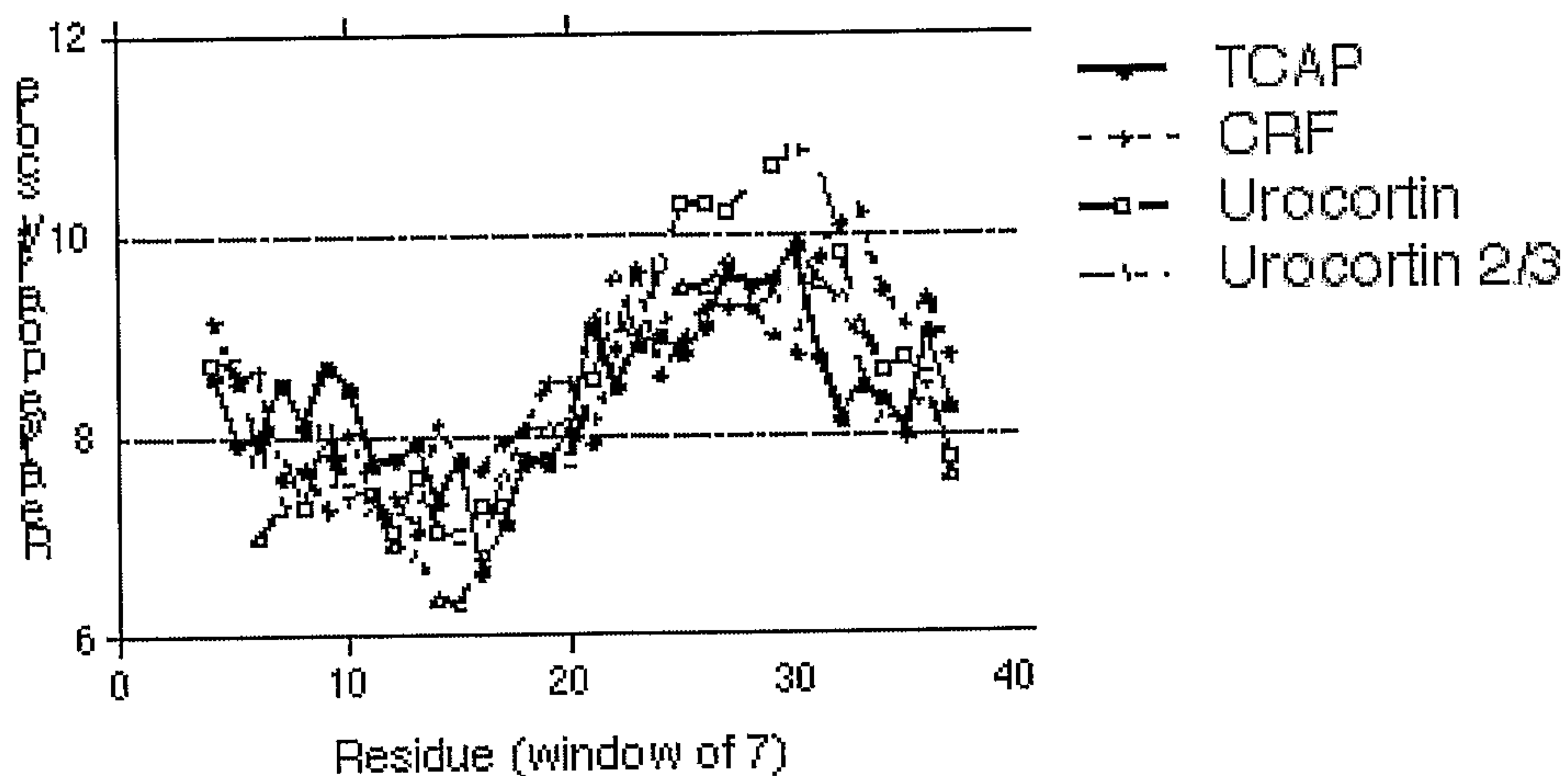
QQLLSTGRVQGYDGYFVLSVEQYLELS DSANNHFMROSEI
 QQLLSTGRVQGYGYVLPVEQYPELA DSSNIOFLRQNEM
 QQLSAGKVQGYDGYVLSVEQYPELA DSANNIOFLRQSEI
 QQLLSTGRVQGYDGYFVLSVEQYPELS DSANNHFMROSEM

FIGURE 9

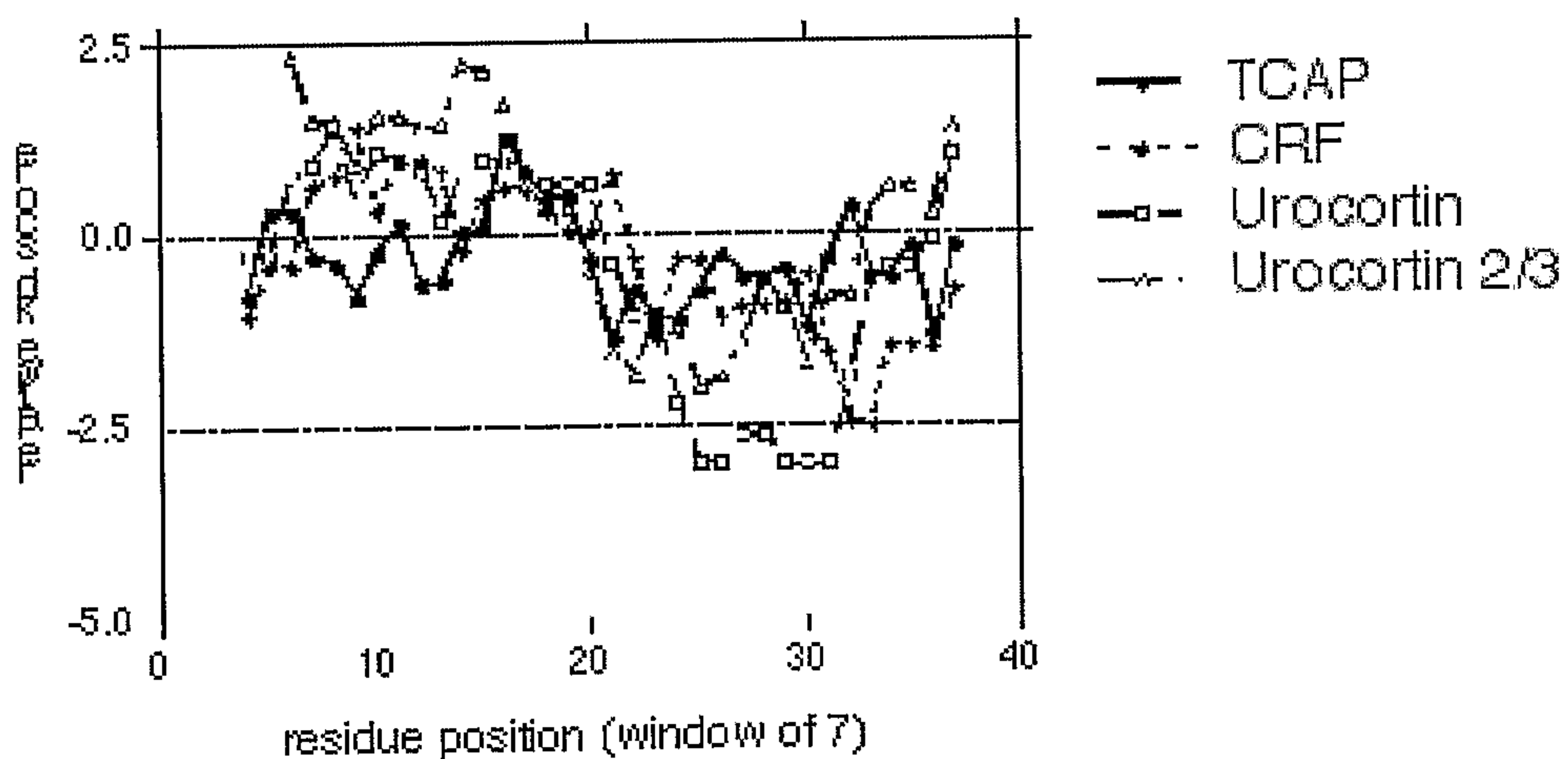
13/30

FIGURE 10

Grantham Polarity Prediction



Kyte-Doolittle Hydrophobicity Prediction



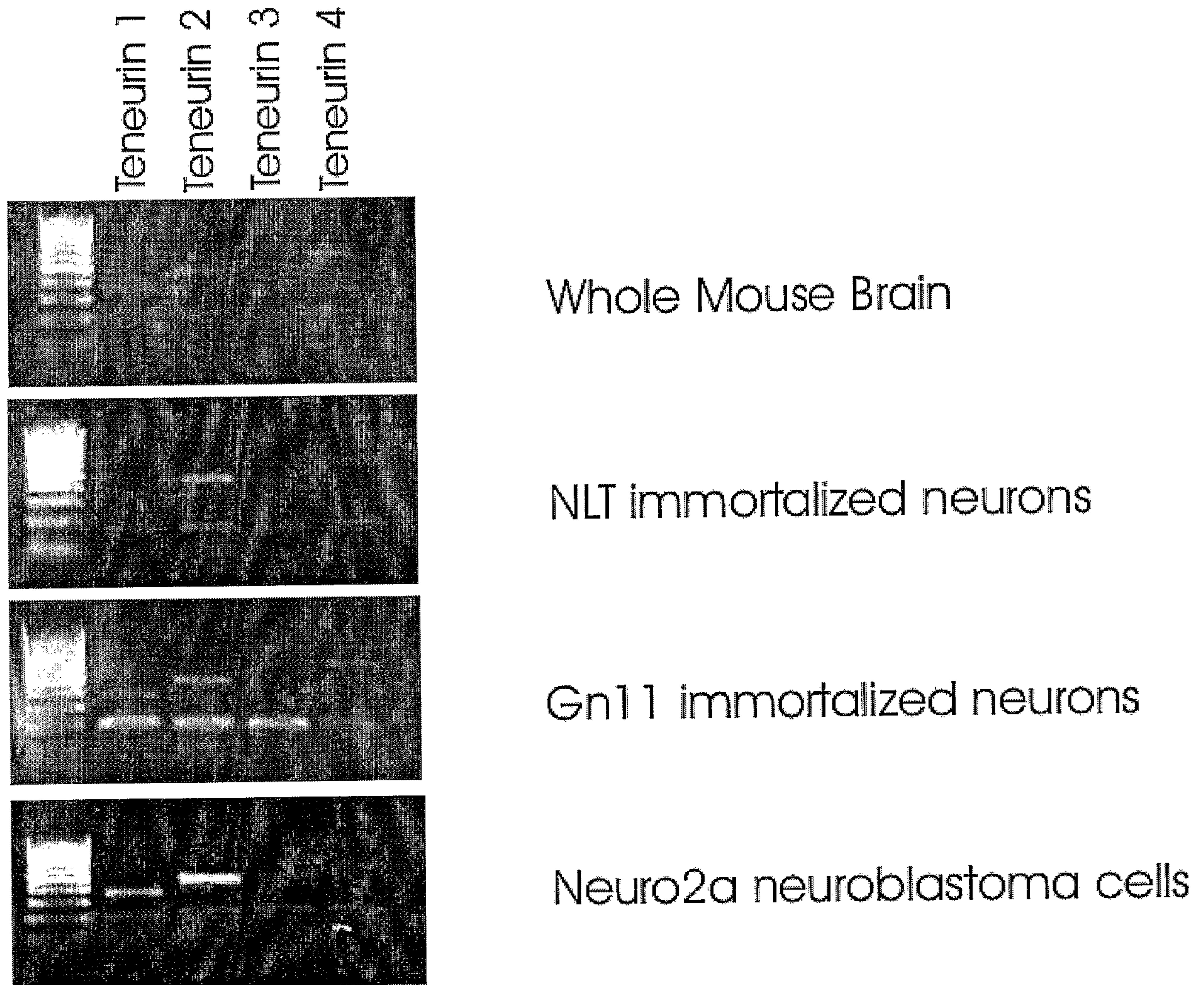
QLLS GRKVLGYDGYVLSIEQYPE LAPS ANNIQFLRQSEI-NH2
 QQLS TGRVQGYDGYFVLSMEQYLE LSDS ANNIHFMRQSEI-NH2
 MGMGPSLSIVNPMVDVLRQR LLLEIARRR LRDAEEQI KANKDFL QQI-NH2
 TGAQSL SIVAPLDVLRQR LMNELNRRR MRELQGSRIQQNRQIL ISI-NH2
 SPTISITAPIDVLR KTWEQERARKQMVVA QNNREFL NSLN-OH
 RMPSSL SIDLPMSVLRQK LSSEKERKVVHALRA AANRNFEL NDI-NH2
 SLSVNPAVDLQHR YMEKVA QNNRNFEL NRW-NH2
 TGSGPSLSIVNPLDVLRQR LLEIARRR MRSQDQI QNRFFL QTI-NH2
 SEEPPSLDLTFHLLR EVLEMARAEQ LAQQ AHSNRKIM EII-NH2
 SDDPPSLDLTFHMLR QMNEMSRAEQ LQQQ AHSNRKMM EIF-NH2
 DDPPLSIDLTFHLLR TLELARTQS QRER AENRIIF DSV-NH2
 QGPPSIDLSLELLR KMIEIEKQEK EKQQ AANNRLL DTL-NH2
 NDDPPSIDLTFHLLR NMIEMARNEN QREQ AGLNRKYL DEV-NH2
 VLLSLDVPIGLLR LLEQARYKA ARNQ AATNAQIL AHV-NH2
 LTLSLDVPTNLMN MLFDVAKAKN LRRAK AAENARLL AHI-NH2
 FTLSLDVPTNLMN LLENIKAKN LRAQ AAANAHELM AQT-NH2

O mykiss TCAP-3 (SEQ.ID.NO.13)
 R. danio TCAP-3 (SEQ.ID.NO.22)
 L. migratoria DP (SEQ.ID.NO.108)
 A. domesticus DP (SEQ.ID.NO.109)
 T. molitor DP (SEQ.ID.NO.110)
 M. sexta DP-I (SEQ.ID.NO.111)
 M. sexta DP-II (SEQ.ID.NO. 112)
 P. Americana (SEQ.ID.NO.113)
 R. norvegicus CRF (SEQ.ID.NO.104)
 O. keta CRF (SEQ.ID.NO.114)
 R. norvegicus UCN (SEQ.ID.NO.115)
 P. sauvageii SVG (SEQ.ID.NO.116)
 C. carpio UI (SEQ.ID.NO.117)
 M. musculus UCN2 (SEQ.ID.NO.118)
 R. danio UCN2 (SEQ.ID.NO.119)
 H. sapiens UCN3 (SEQ.ID.NO.107)

FIGURE 11

15/30

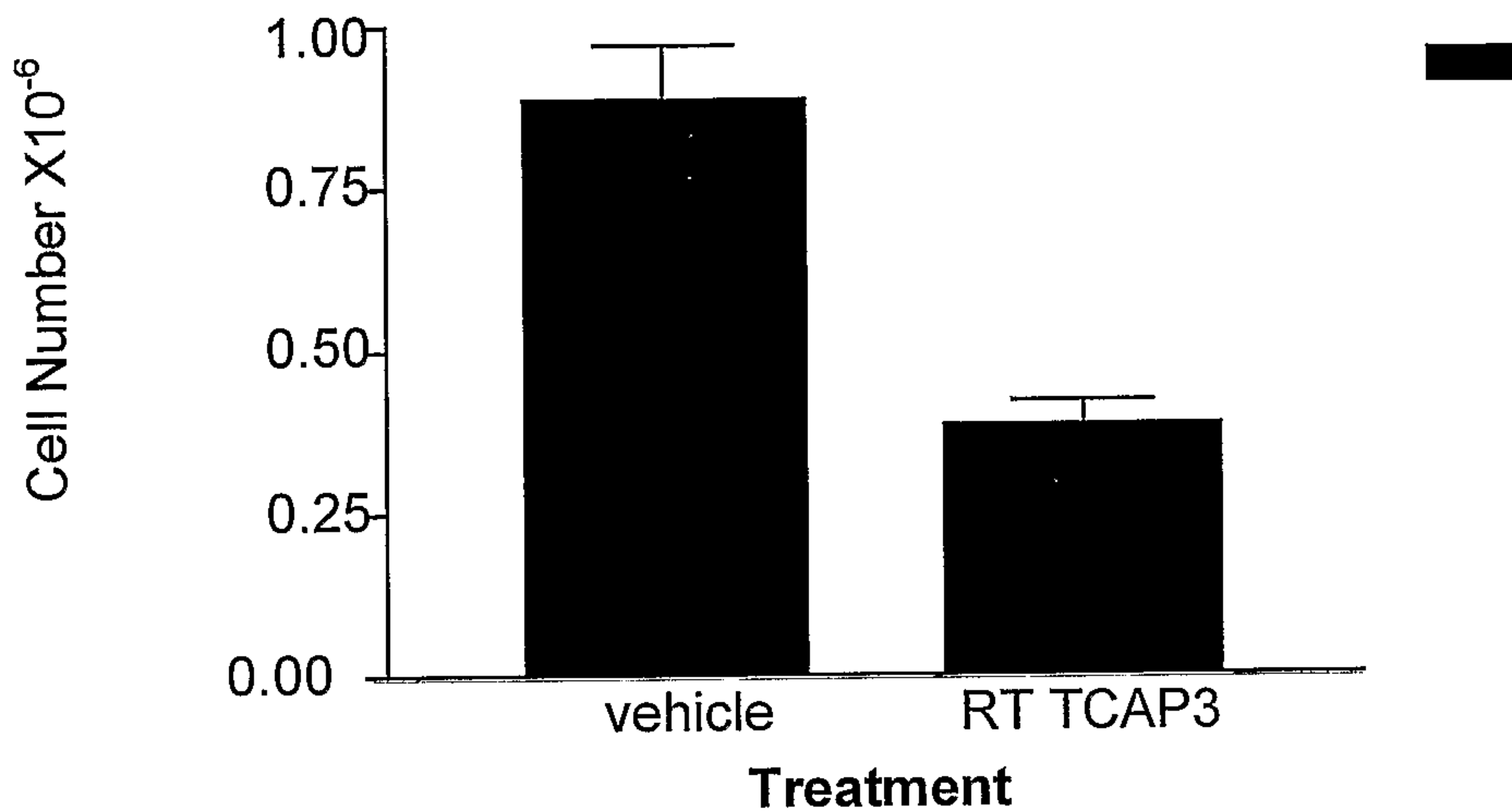
Figure 12



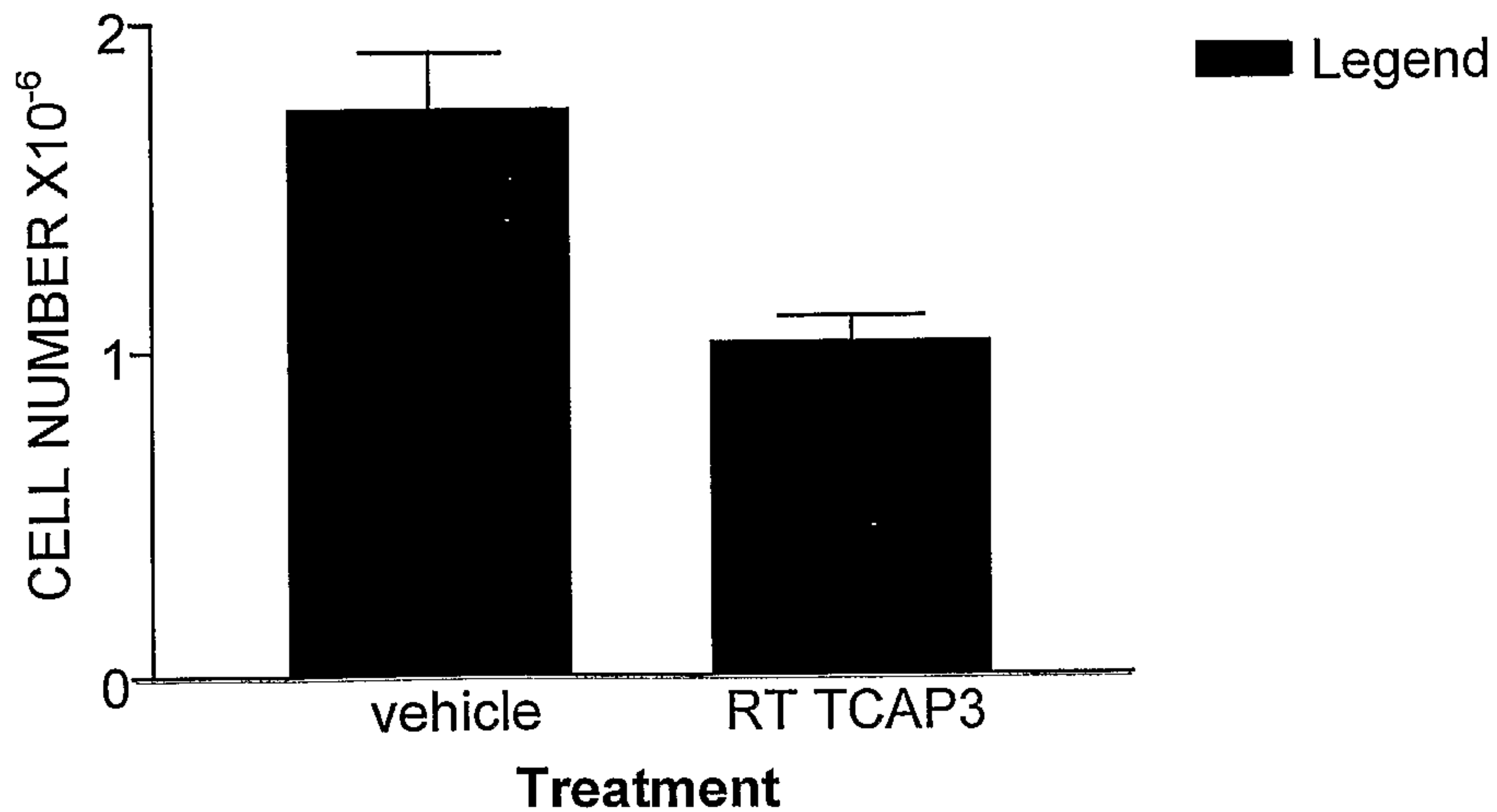
16/30

FIGURE 13

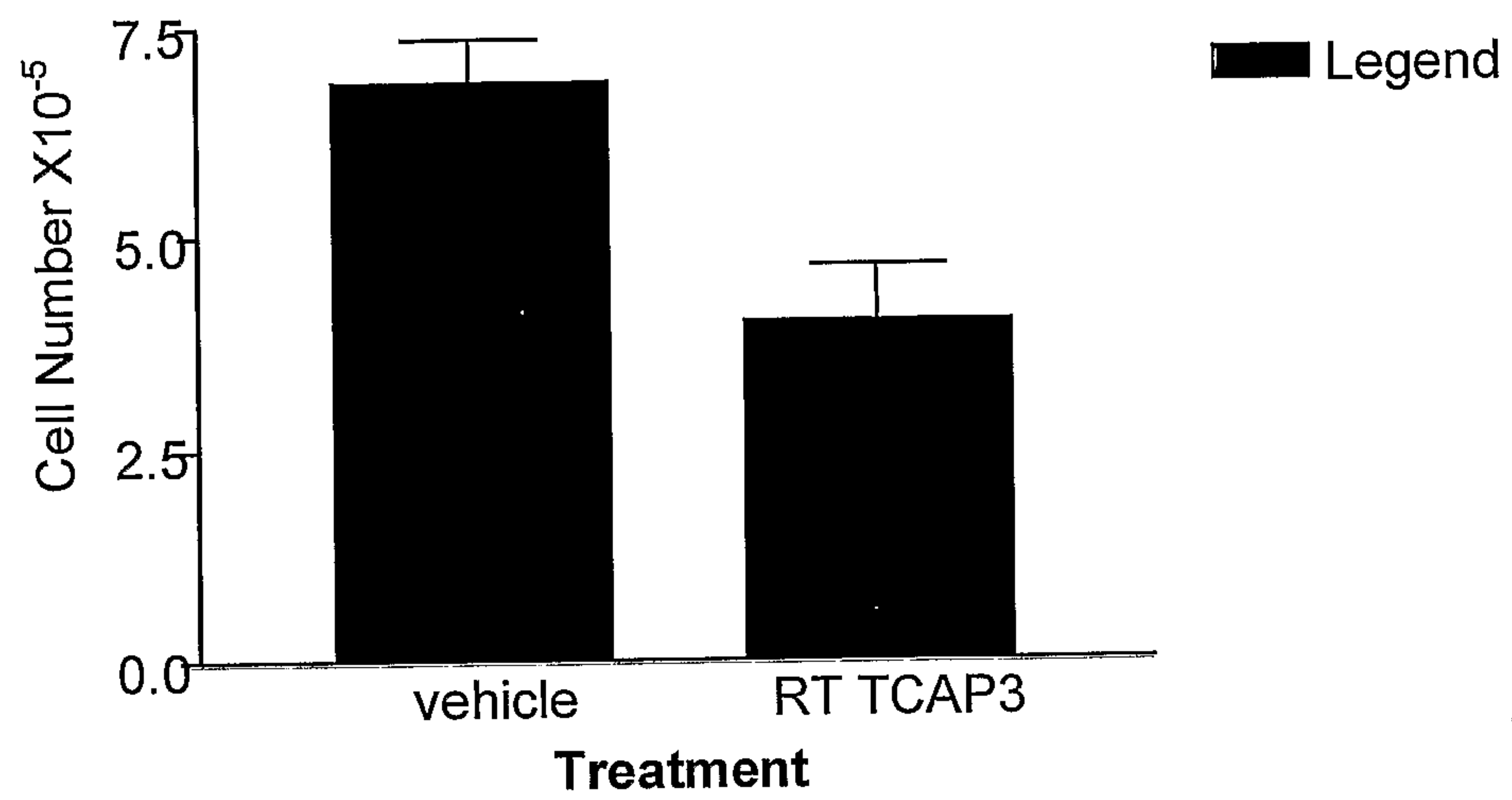
Effect of 10^{-6} M RT TCAP3 on Gn11 cells at 48-hrs



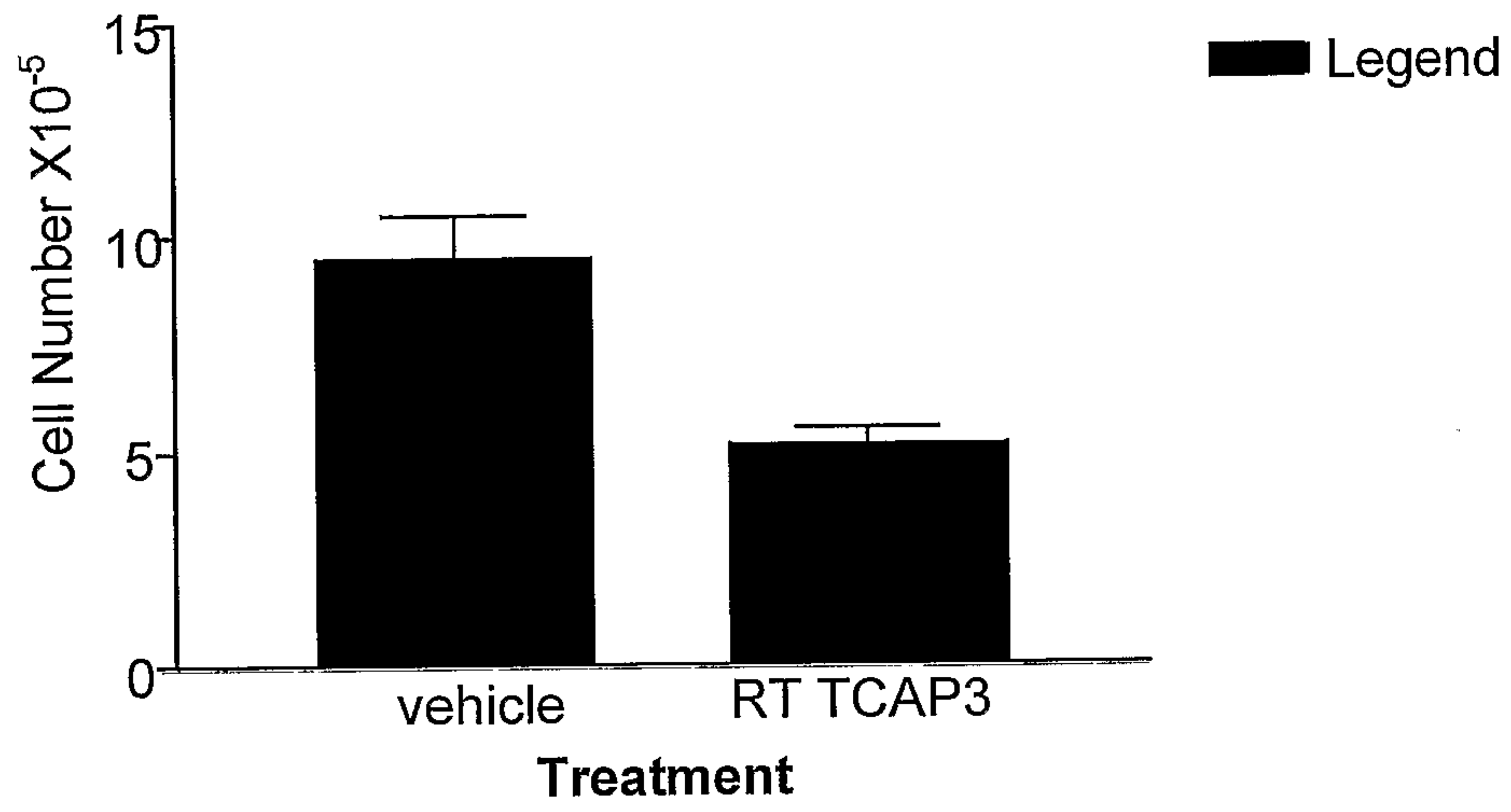
Effect of 10^{-6} M RT TCAP3 on Gn11 cells at 72-hrs



17/30

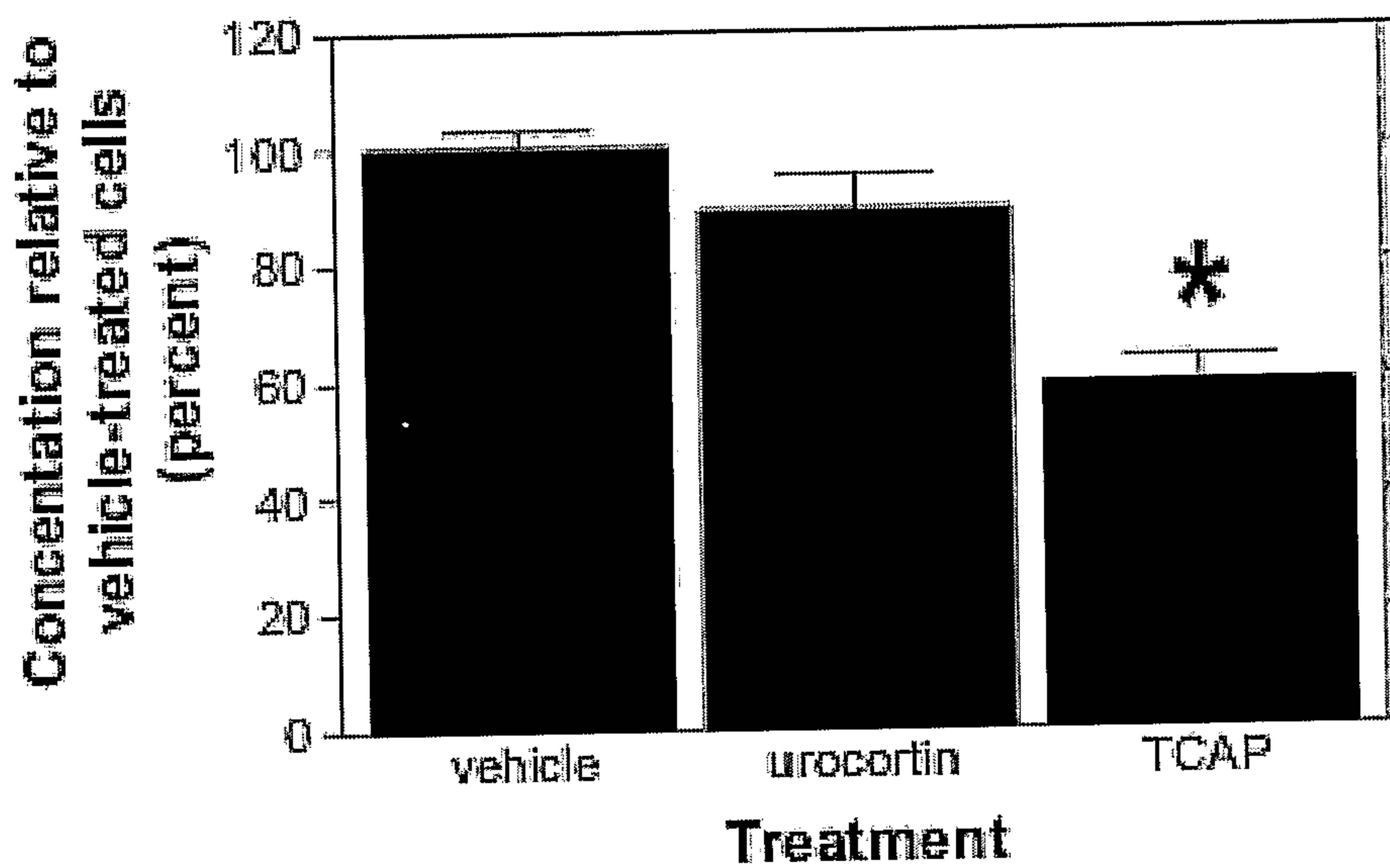
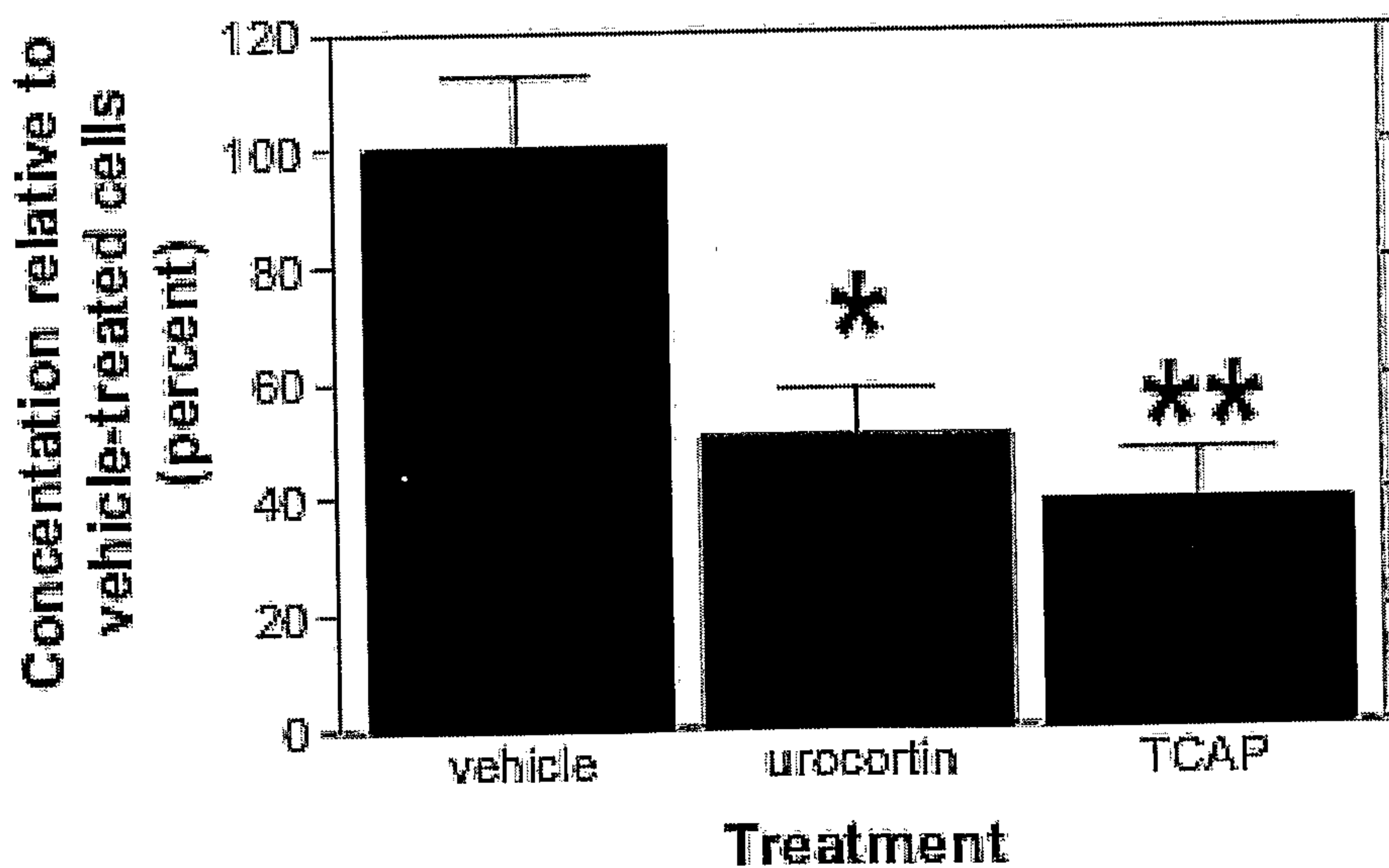
FIGURE 14**Effect of 10^{-6} M RT TCAP3 on TGR1
cells at 48-hrs**

18/30

FIGURE 15**Effect of 10^{-6} M RT TCAP3 on
HO16.4c cells at 48-hrs**

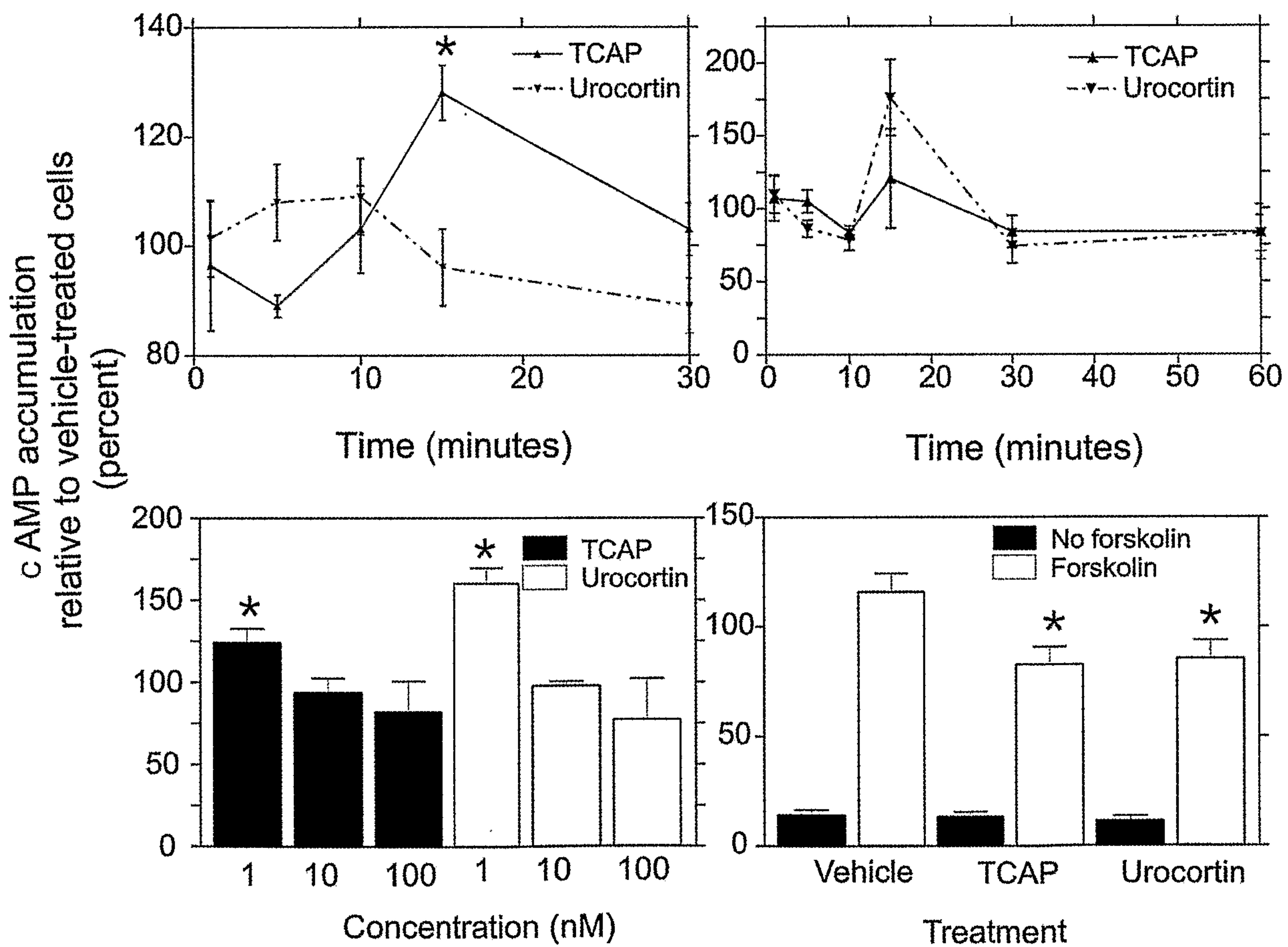
19/30

FIGURE 16

A CAMP**B cGMP**

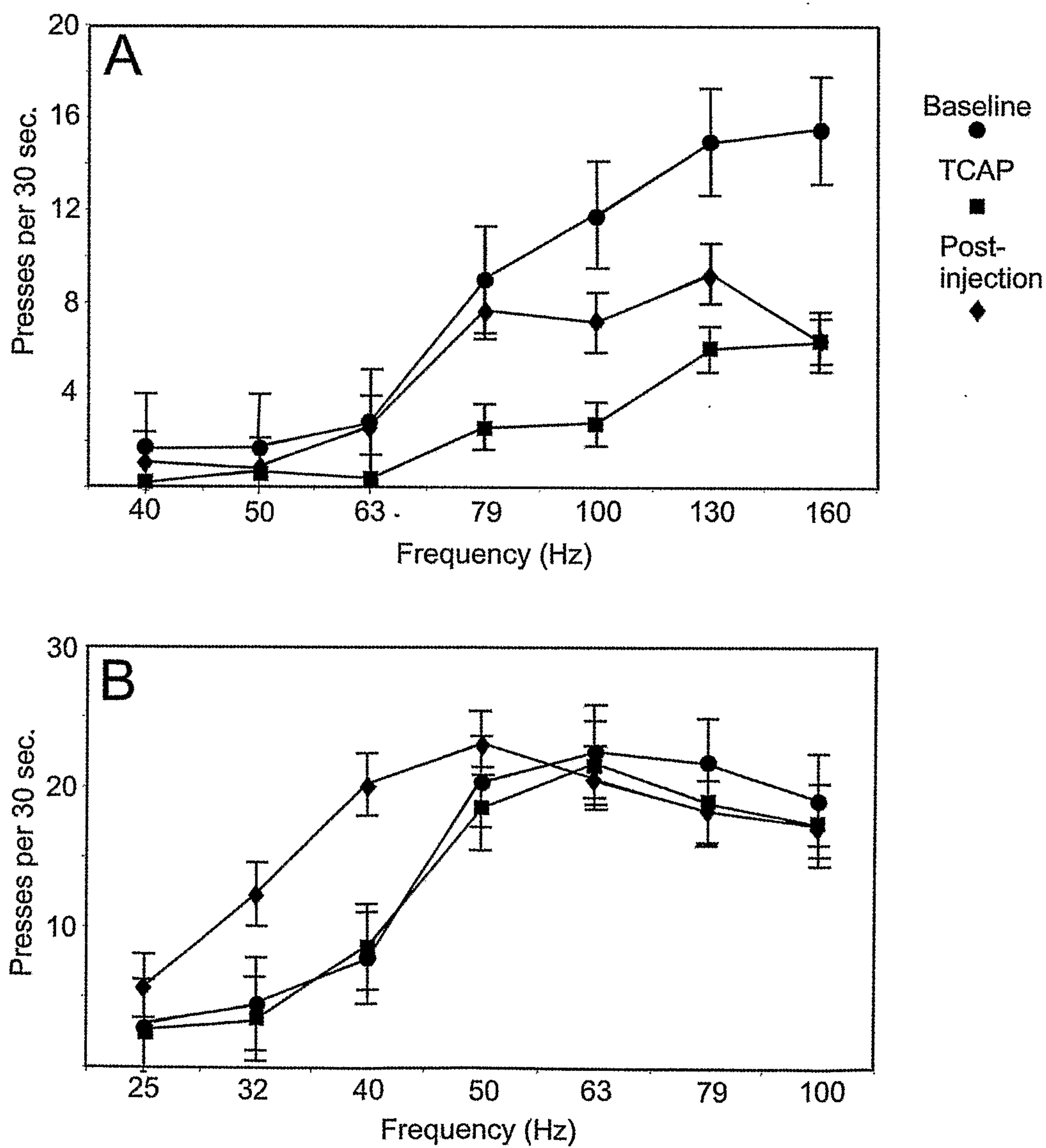
20/30

FIGURE 17



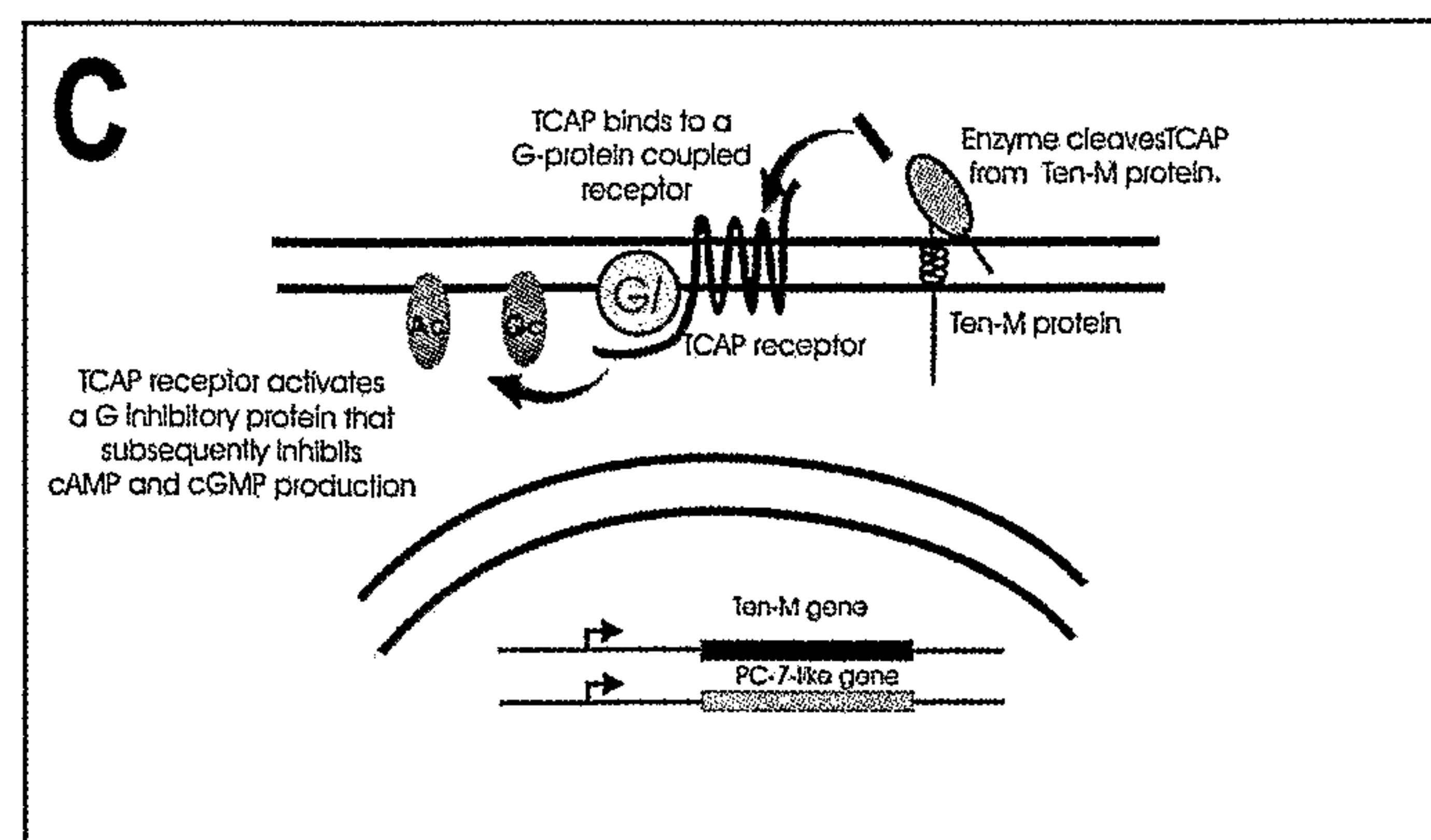
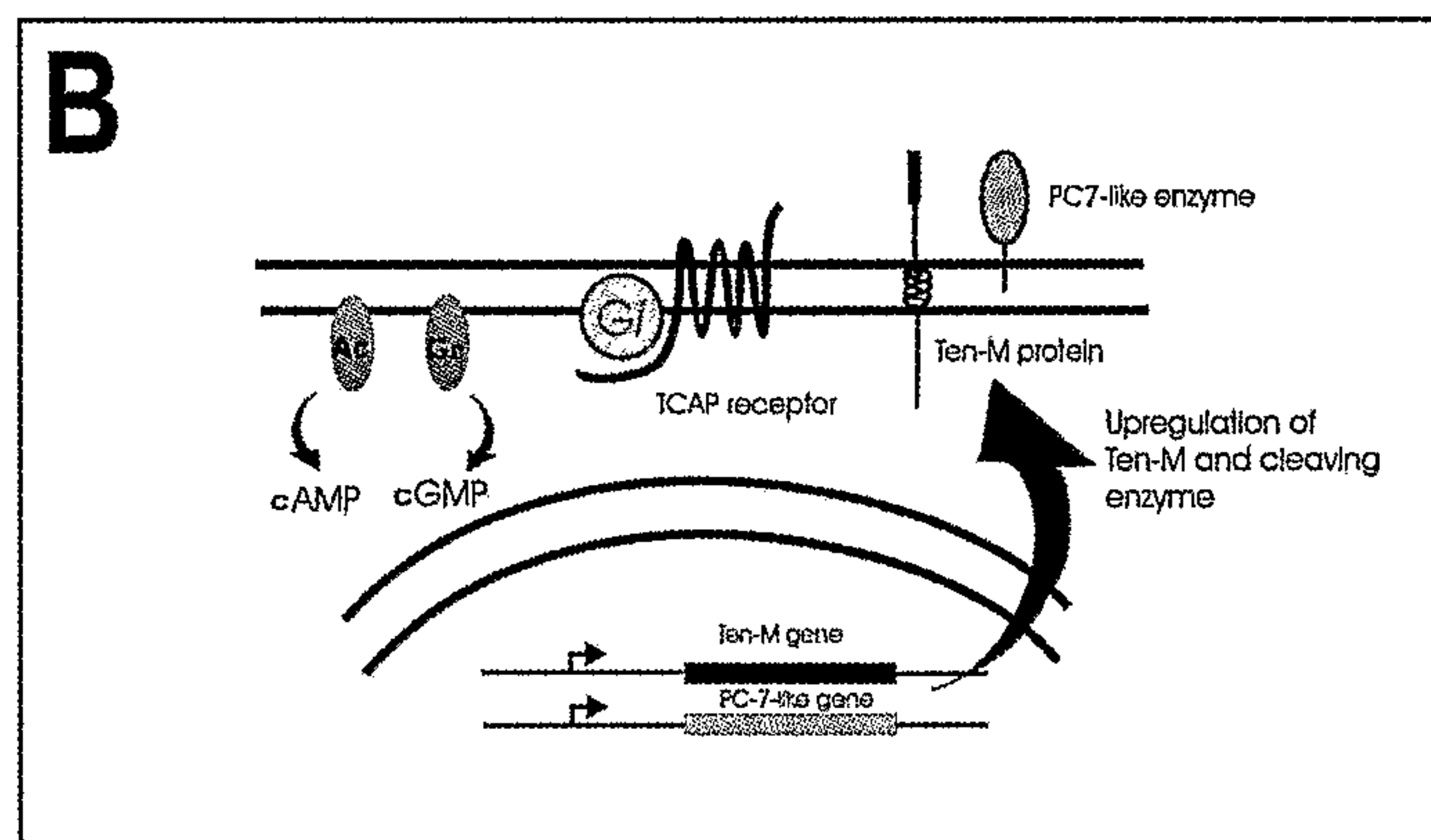
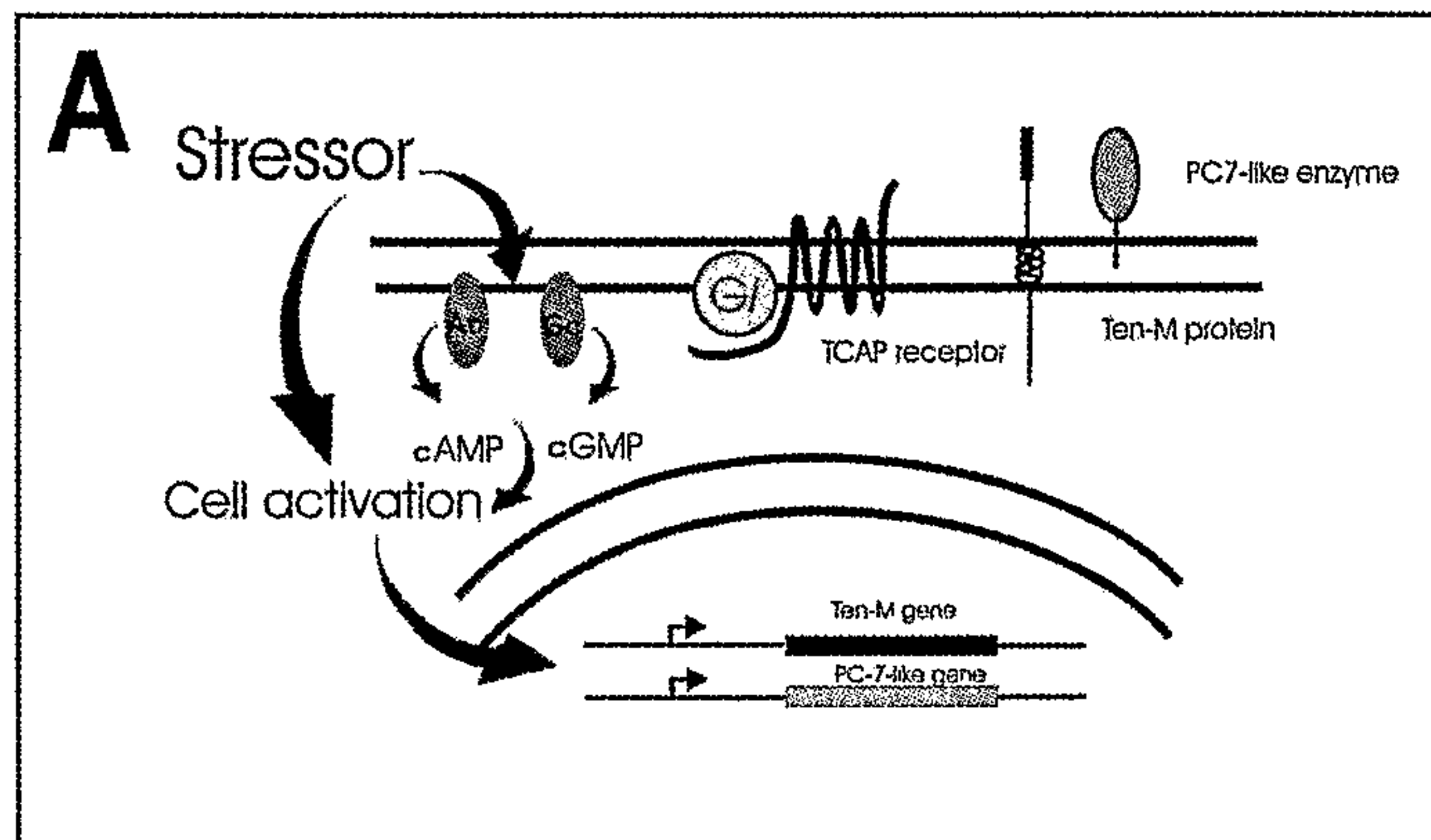
21/30

FIGURE 18



22/30

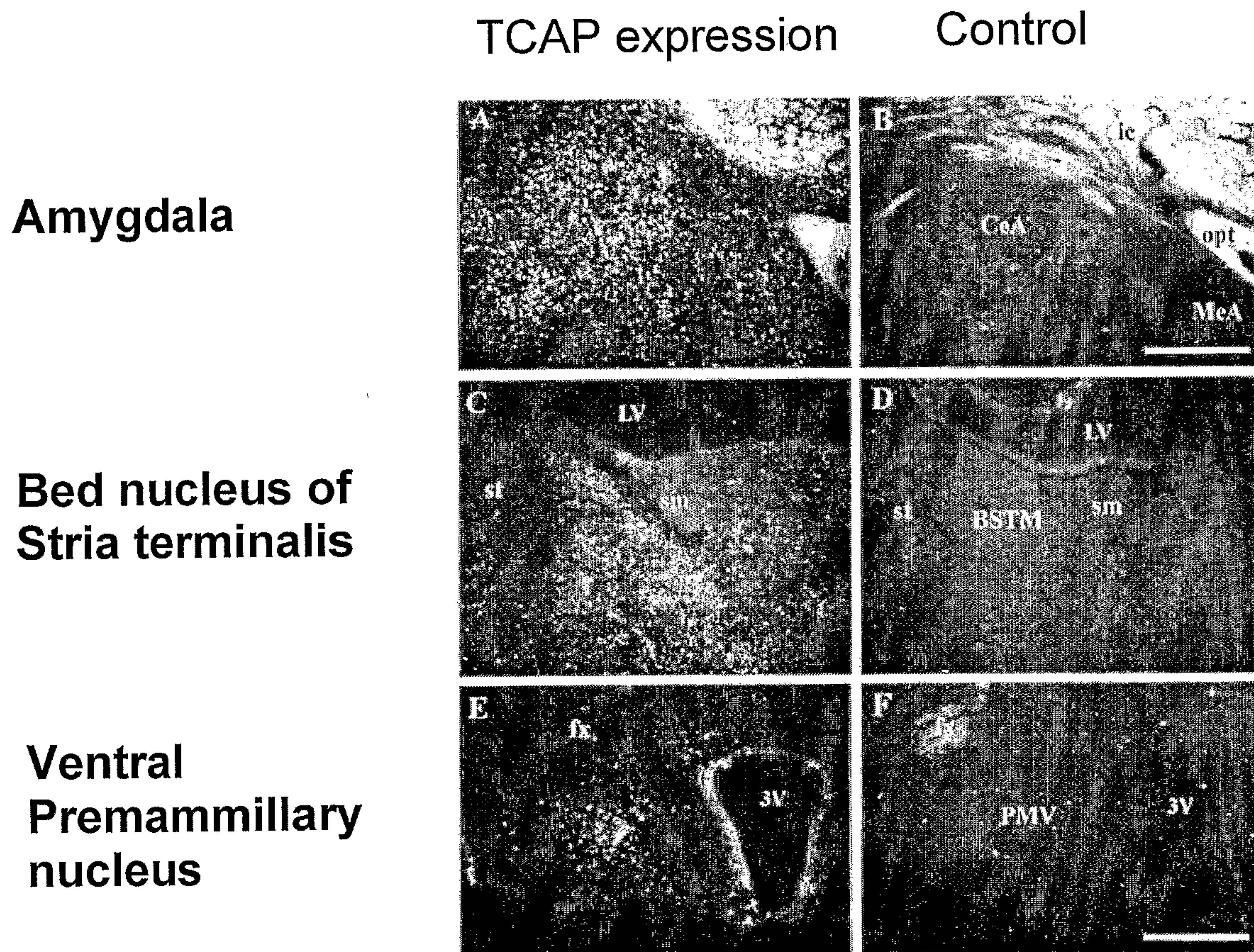
FIGURE 19



23/30

FIGURE 20

In Situ Hybridization



Vehicle-Treated Rats-ICV injected

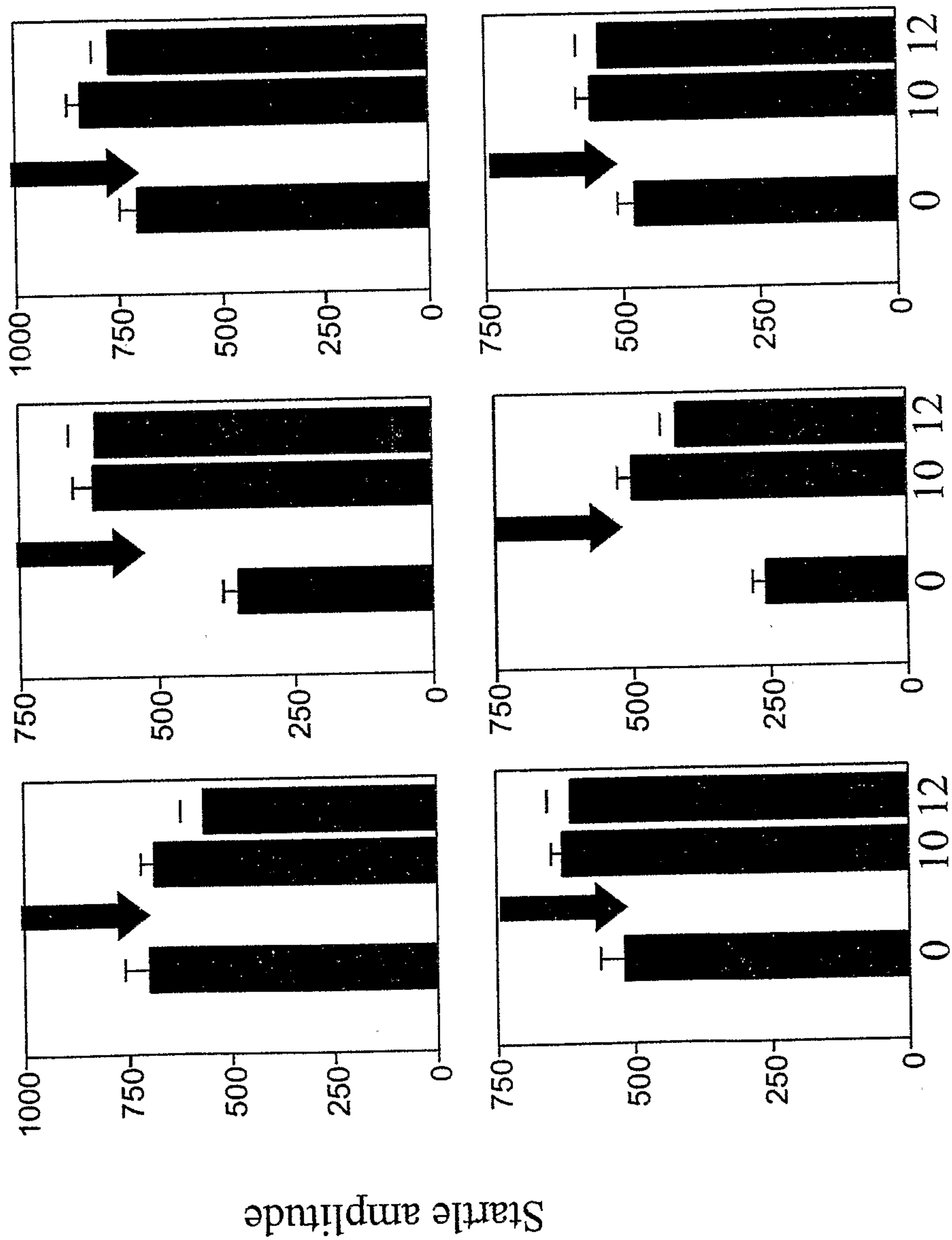


FIGURE 21A

TCAP-1 Treated Rats-ICV injected

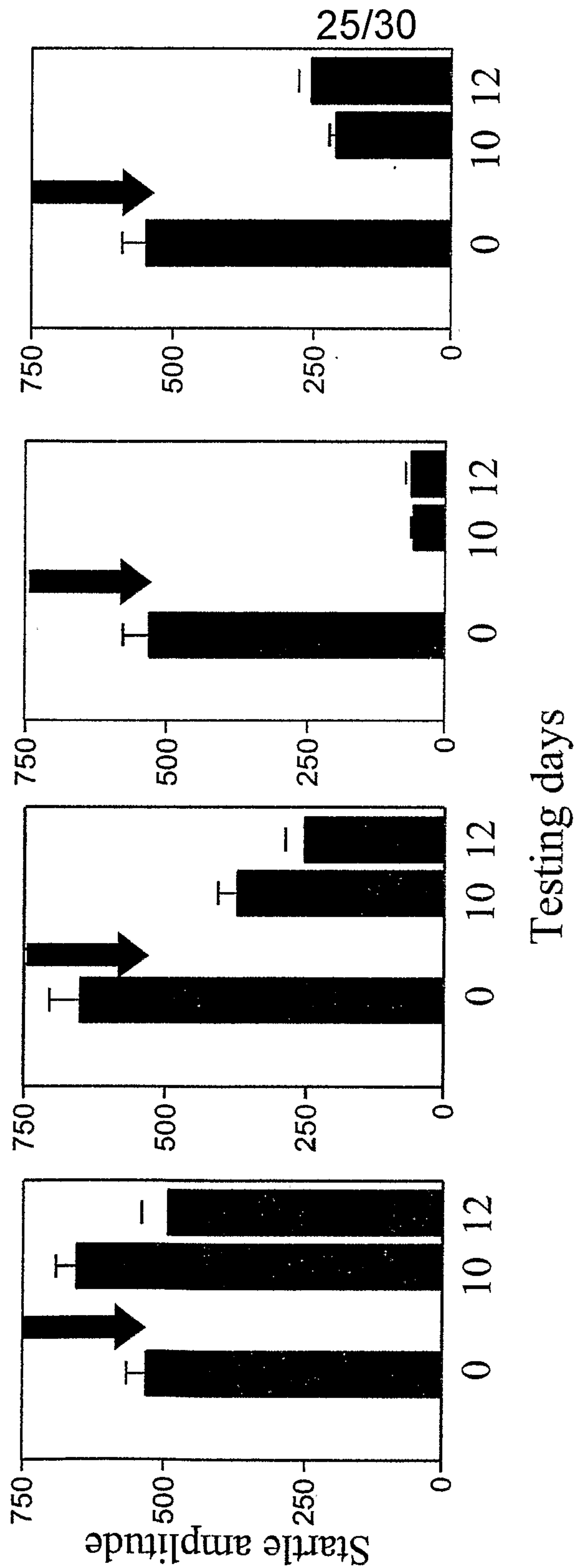
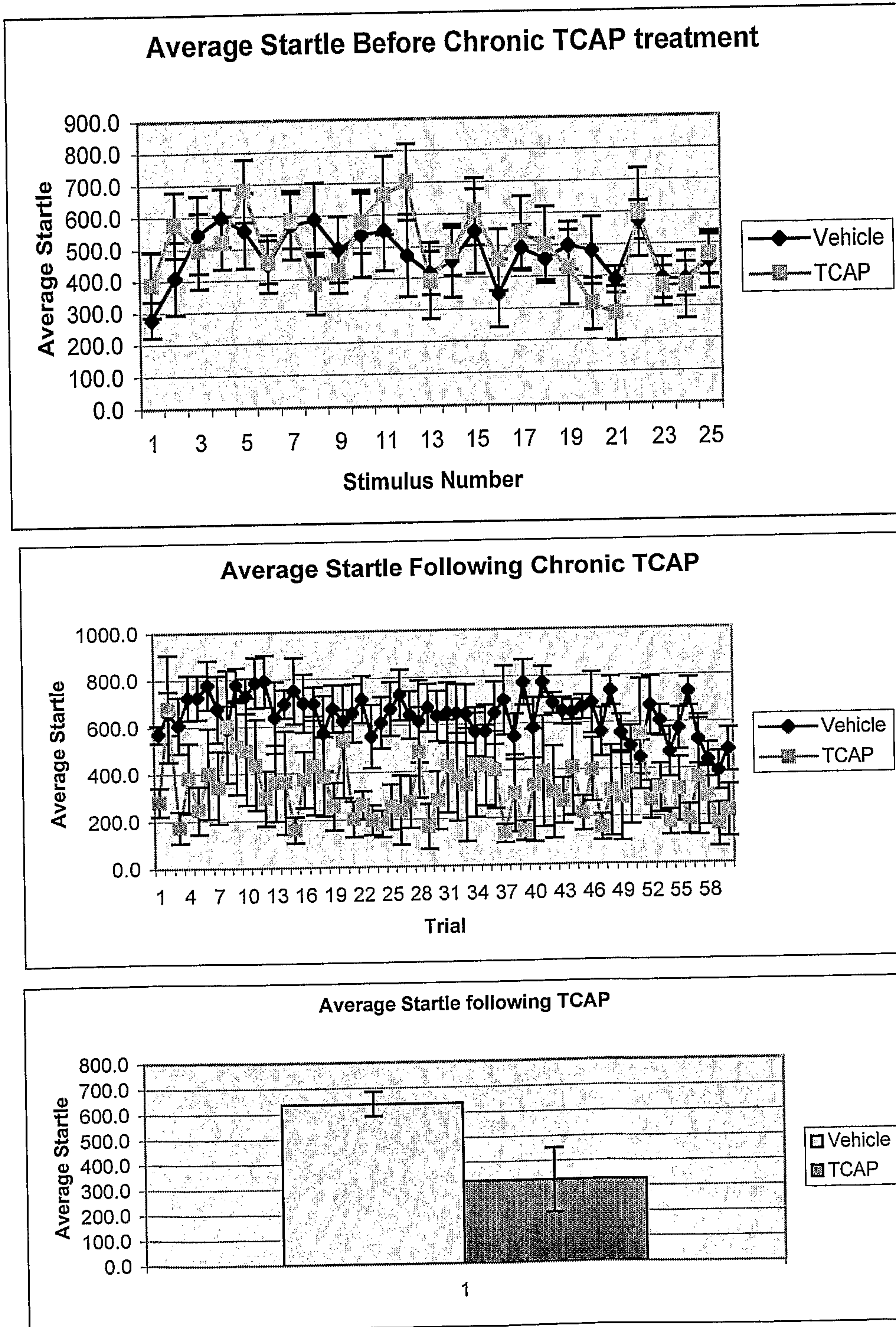


FIGURE 21B

FIGURE 22



27/30

FIGURE 23

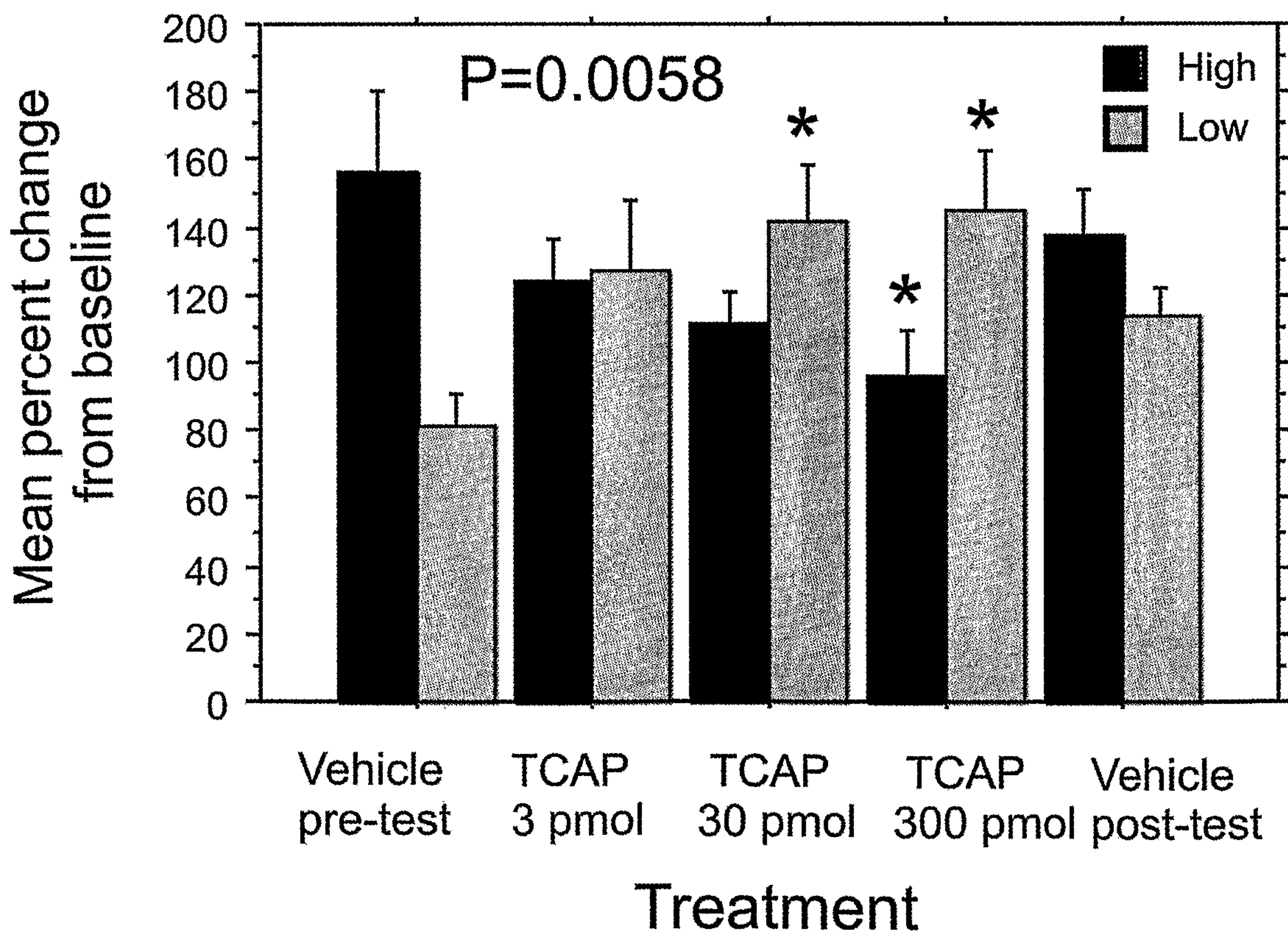
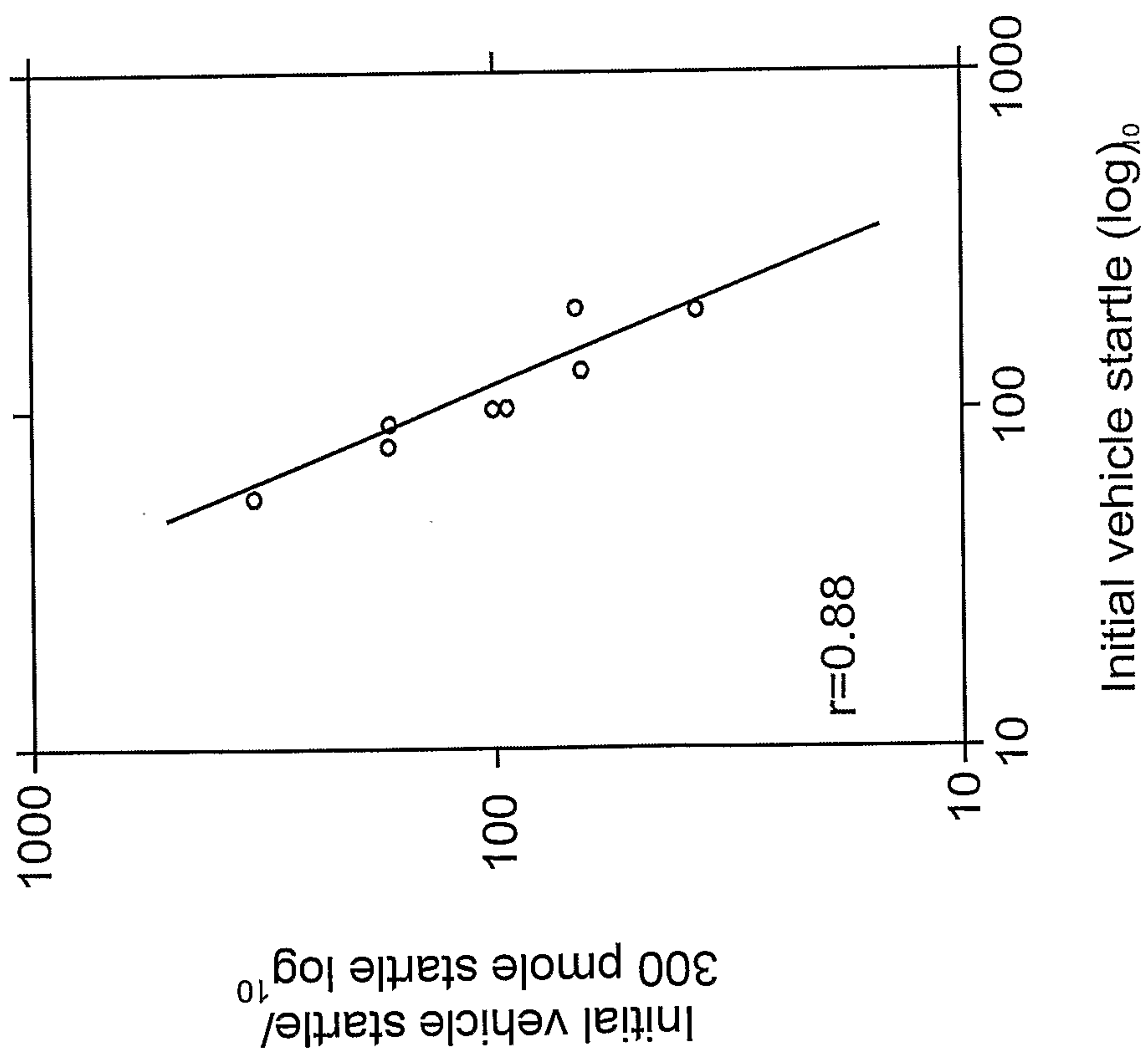
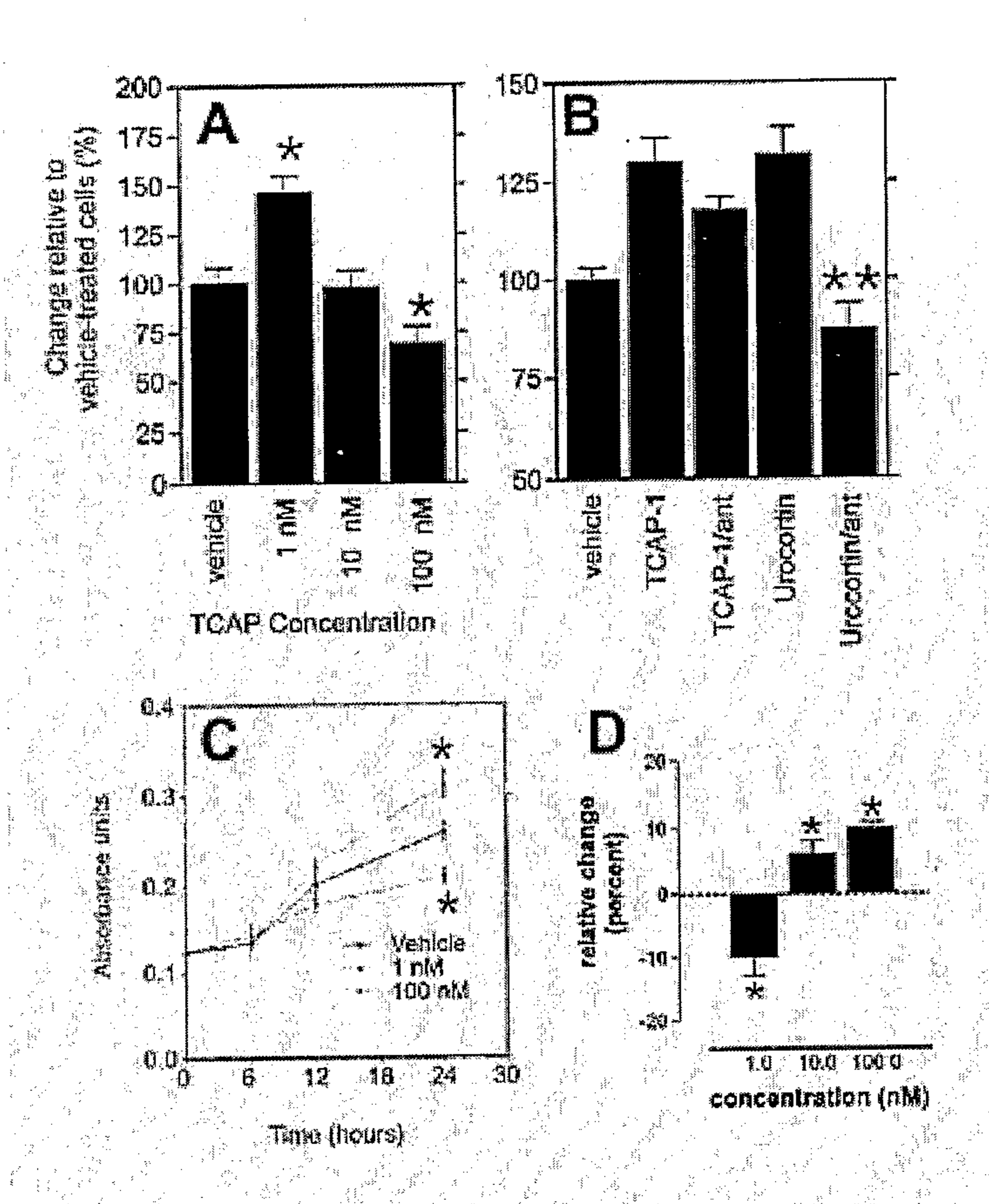


FIGURE 24
Summary of amygdala-injected TCAP-1



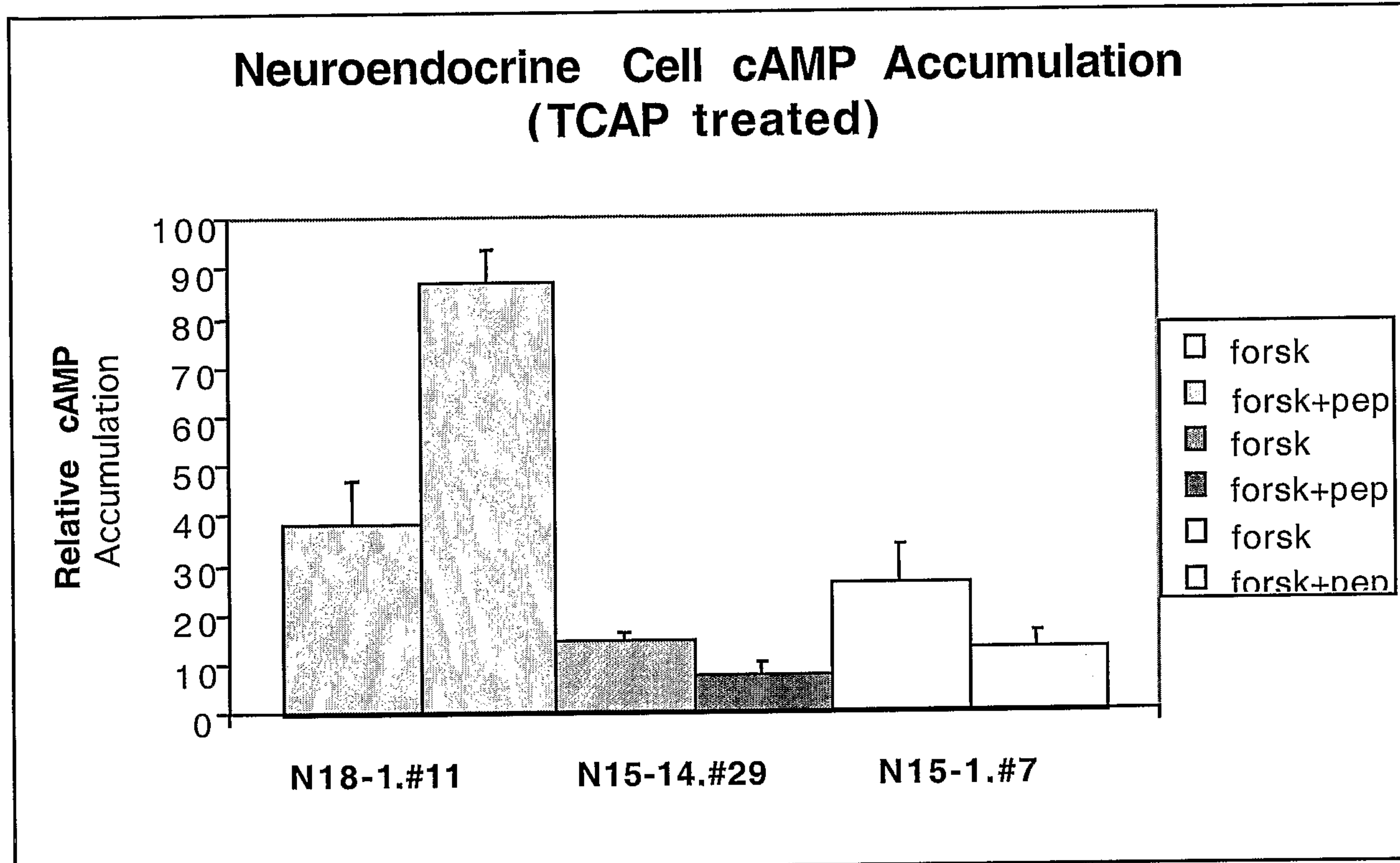
29/30

FIGURE 25



30/30

FIGURE 26



Number of residues

