Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9633–9637, October 1995
Biochemistry

Thyroid-stimulating hormone (TSH)-directed induction of the CREM gene in the thyroid gland participates in the long-term desensitization of the TSH receptor

(gene regulation/transcriptional repression/inducible response/pituitary)

ENZO LALLI AND PAOLO SASSONE-CORSI*

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique–Institut National de la Santé et de la Recherche Médicale, BP163, Illkirch 67404, CU de Strasbourg, France

Communicated by Pierre Chambon, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France, July 7, 1995

ABSTRACT **Thyroid gland function is regulated by the hypothalamic–pituitary axis via the secretion of TSH, according to environmental, developmental, and circadian stimuli. TSH modulates both the secretion of thyroid hormone and gland trophism through interaction with a specific guanine nucleotide-binding protein-coupled receptor (TSH receptor; TSH-R), which elicits the activation of the cAMP-dependent signaling pathway. After TSH stimulation, the levels of TSH-R RNA are known to decrease dramatically within a few hours. This phenomenon ultimately leads to homologous long-term desensitization of the TSH-R. Here we show that TSH drives the induction of the inducible cAMP early repressor (ICER) isoform of the cAMP response element (CRE) modulator gene both in rat thyroid gland and in the differentiated thyroid cell line FRTL-5. The kinetics of ICER protein induction mirrors the down-regulation of TSH-R mRNA. ICER binds to a CRE-like sequence in the TSH-R promoter and represses its expression. Thus, ICER induction by TSH in the thyroid gland represents a paradigm of the molecular mechanism by which pituitary hormones elicit homologous long-term desensitization.**

Thyroid gland function is regulated by the hypothalamic–pituitary axis via the secretion of thyrotropin (thyroid-stimulating hormone; TSH), according to environmental, developmental, and circadian stimuli (1, 2). TSH modulates both the secretion of thyroid hormone and gland trophism through interaction with a specific receptor on the thyroid cell membrane (TSH receptor; TSH-R; ref. 3). The TSH-R belongs to the guanine nucleotide-binding protein (G protein)-coupled, seven-transmembrane-domain receptor family and presents regions of homology with the other receptors for pituitary glycoprotein hormones (4). The cAMP signal-transduction pathway is essential in mediating signaling through the TSH-R (4). cAMP, in turn, interacts with the regulatory subunit of protein kinase A (PKA), releasing the catalytic subunit from constitutive inhibition and allowing phosphorylation of multiple substrates residing both in the cytoplasm and in the nucleus (5).

TSH-mediated triggering of the cAMP signal-transduction pathway in thyroid cell elicits the activation of genes such as thyroglobulin and thyroid peroxidase, which are involved in the production of thyroid hormone (6). cAMP inducibility of these genes has been shown to be dependent on the homeodomain transcription factor thyroid transcription factor 1 (TTF-1), whose DNA-binding activity is stimulated by phosphorylation by PKA (7–10). In addition to the stimulation of these genes, the expression of other genes in the thyroid gland is repressed by TSH. The best known example is represented by the TSH-R (11). Down-regulation of the TSH-R gene is crucial in the attenuation process of the thyroid cell response to TSH. Using this negative feedback mechanism (long-term desensitization; LTD), it is possible to avoid excessive hormone production and gland hypertrophia after TSH stimulus. In fact, inappropriate stimulation in the production of cAMP in the thyroid leads to hyperthyroidism and goiter formation. This is demonstrated by the recent finding of activating mutations in the coding sequence of the human TSH-R in some cases of congenital hyperthyroidism and thyroid adenoma (12, 13).

Importantly, it has been shown that transcriptional down-regulation represents the molecular basis for TSH receptor LTD in thyroid cells (11). Here we show that TSH treatment drives cAMP-response element modulator (CREM) expression in the rat thyroid gland and in the differentiated thyroid cell line FRTL-5. The TSH-induced CREM isoform corresponds to inducible cAMP early repressor (ICER), a small protein encoded by an alternative, intronic promoter in the CREM gene. ICER is known to be a powerful transcriptional repressor. ICER displays a characteristic early response inducibility in thyroid cells, which correlates with TSH-R transcriptional down-regulation. We show that ICER represses the expression of TSH-R. Thus, CREM inducibility may represent a paradigm of the molecular mechanism used by pituitary hormones to obtain homologous LTD of their own receptor.

MATERIALS AND METHODS

Cell Culture. FRTL-5 rat thyroid cells were cultured as described (14). JEG-3 human choricarcinoma cells were grown in Dulbecco's modified Eagle's medium, supplemented with 10% (vol/vol) fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 mg/ml.

Animals and Drug Treatments. Hypophysectomized and normal 9-week-old Fischer 344 male rats were obtained from Iffa-Credo (L'Anbresle, France). For hormonal stimulations, hypophysectomized animals were injected i.p. with 1 unit of bovine TSH, ovine follicle-stimulating hormone (FSH), or sheep prolactin. Groups of normal rats were treated with methimazole (0.05% in drinking water) or daily injected with triiodothyronine (T_3; 100 μg/100 g of body weight). Both treatments lasted for 8 days. The efficacy of the treatment was confirmed by assay of free T_3 and thyroxine in rat serum. All hormones and drugs were obtained from Sigma.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PKA, protein kinase A; CRE, cAMP response element; CREM, CRE modulator; ICER, inducible cAMP early repressor; TSH, thyroid-stimulating hormone; TSH-R, TSH receptor; LTD, long-term desensitization; G6PD, glucose-6-phosphate dehydrogenase; RT, reverse transcription; G protein, guanine nucleotide-binding protein; FSH, follicle-stimulating hormone; TTF-1, thyroid transcription factor 1; T_3, triiodothyronine.

*To whom reprint requests should be addressed.

RNase Protection. RNA was extracted from rat organs by the guanidinium thiocyanate method (15). RNase protection was performed as described (16, 17). Equivalent loading was confirmed by glucose-6-phosphate dehydrogenase (G6PD) reverse-transcribed PCR internal controls (16).

Western Analysis. Thyroid glands dissected from rats immediately after cervical dislocation were directly homogenized in Laemmli buffer. Western blotting was performed as described (18), using an affinity-purified rabbit antiserum raised against bacterially produced CREMτ protein. This antiserum shows minimal crossreactivity to cAMP-response element (CRE) binding protein (18).

Reverse Transcription (RT)-PCR. One microgram of RNA obtained from 5H and 6H FRTL-5 cells and from the thyroid glands of hypophysectomized rats was reverse-transcribed, and cDNA was amplified as described (19). Use of the same quantity of cDNA for TSH-R amplification was verified by G6PD RT-PCR. For TSH-R amplification, primers AGCACCCAGACTCTGAAGCT and GGTTTCATTGCATAGTCCCTG were used, which amplify a 380-bp fragment. For G6PD amplification, primers ATCTACCGCATTGACCACTACCTG and CCCACAGAAGACATCCAGGATGAG were used, which amplify a 756-bp fragment (16). For both TSH-R and G6PD, PCR conditions were 30 cycles of 30 sec at 94°C, 1 min at 60°C, and 45 sec at 72°C. PCR products were transferred to a Hybond N^+ nylon membrane (Amersham). The blot was hybridized with a full-length human TSH-R cDNA probe and with the internal G6PD oligonucleotide TTCATCCTGCGCTGTGGCAAAGCTCTGAATGAGCGCAAAGCTGAA.

Gel Mobility Shift Assays. Gel retardation assays were performed as described (20), using as probes the −199/+1 TSH-R promoter DNA sequence (excised from plasmid pGE-T200 by *Sac* I–*Hin*dIII digestion) and a double-stranded oligonucleotide containing the TSH-R promoter −146/−114 (relative to the translation start site) sequence (CCAGCGATGAGGTCACAGCCCCTTGGAGCCCTC; the underlining indicates the CRE sequence). For competition experiments, in addition to cold −146/−114 oligonucleotide, cold double-stranded oligonucleotides with the somatostatin CRE (AGCTCCGCCCAGTGACGTCAGAGAGA) and the heat shock transcription factor (AGCTTGAGAAACAAGAACATTCGGGTGGTTT) binding site were used. Bacterially expressed CREM ICER proteins were prepared as described (17), whereas a small-scale method was used to prepare FRTL-5 cell nuclear extracts (21).

Cell Transfections. JEG-3 cells were transfected as described (20), by the calcium phosphate technique. As a reporter plasmid, pGE-T200 (gift of D. Civitareale, Maris Negri Institute) was used, which contains the −199/+1 TSH-R promoter sequence cloned upstream of the luciferase gene (22). As expression vectors, we used pCH110, which encodes *Escherichia coli* β-galactosidase as a transfection control; pCαEV (gift of G. S. McKnight, University of Washington), which encodes the catalytic subunit of the mouse PKA gene; and pSV-ICERII and pSV-ICERIIγ, which encode the two ICER isoforms comprising or not the CREM-specific γ domain, respectively (17). The luciferase assay was performed on cell extracts as described (23).

RESULTS

CREM Is Expressed at High Levels in the Rat Thyroid Gland. The importance of the cAMP-responsive pathway in the endocrine system and the crucial role played by CREM in the nuclear hormonal response (5) prompted us to analyze the levels of CREM expression in various tissues (Fig. 1*A*). While expression from the P_1-originated transcripts is very low (data not shown), transcripts originating from the intronic, cAMP-inducible P_2 promoter are readily detected at high levels in the thyroid gland (lane 7), as well as in other endocrine tissues such as the pineal and pituitary glands (lanes 4 and 6), as reported earlier (24). The transcripts originating from the P_2 promoter generate the powerful, cAMP-inducible transcriptional repressor ICER (17).

ICER Expression in the Thyroid Is Modulated by TSH. In the pineal gland, ICER expression can be modulated by adrenergic signals from the suprachiasmatic nucleus (24). The molecular mechanism responsible for ICER responsiveness to the adrenergic stimulation involves the presence of four CRE-like promoter elements in the P_2 CREM regulatory region (17).

To investigate the relationship of ICER expression in the thyroid with the principal regulator of thyroid functions, the pituitary hormone TSH, we utilized the model of the hypophysectomized rat. In these animals, production of TSH is suppressed and thyroid glands are hypotrophic. TSH administra-

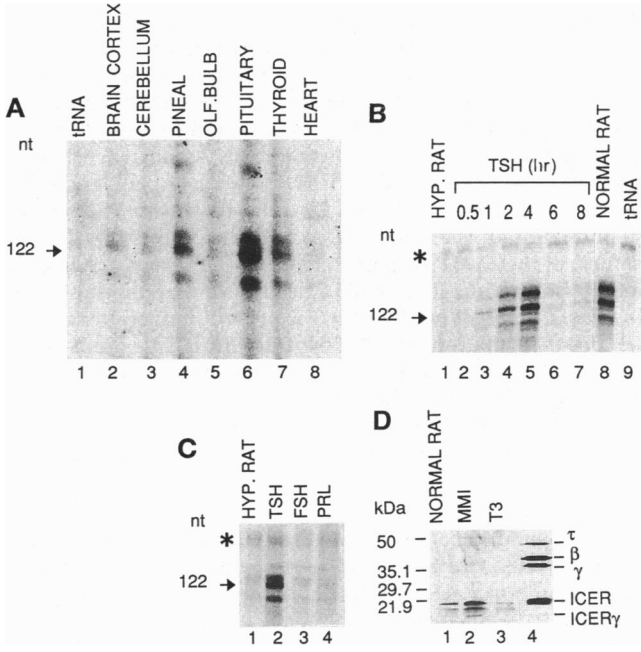

FIG. 1. Pituitary hormone TSH directs CREM expression in the thyroid gland. (*A*) ICER expression in rat tissues. RNase protection analysis of CREM RNA in a panel of rat tissues, using the P75 probe, which specifically recognizes transcripts originating from the P_2 CREM promoter (17). Ten micrograms of total RNA per lane was used; yeast tRNA was used as a negative hybridization control. The size of the protected fragment is indicated (122 nt). OLF., olfactory. (*B*) ICER induction in the thyroid gland by TSH. The time course of CREM expression in the hypophysectomized (HYP.) rat thyroid after i.p. injection of 1 unit of bovine TSH is shown. Ten micrograms of total thyroid RNA per time point was used in a RNase protection assay using the P75 probe. Each lane represents a single animal. Animals were sacrificed before (lane 1) and 0.5, 1, 2, 4, 6, and 8 hr after injection (lanes 2–7). ICER expression in normal rat thyroid is also shown (lane 8). The results have been reproduced in three independent experiments. The asterisk indicates a nonspecific band. (*C*) ICER expression is specifically induced by TSH in rat thyroid. Levels of ICER RNA expression in the thyroid gland of hypophysectomized rats before (lane 1) and 4 hr after i.p. injection of 1 unit of bovine TSH (lane 2), 1 unit of ovine FSH (lane 3), or 1 unit of sheep prolactin (PRL; lane 4) are shown. An asterisk indicates nonspecific hybridization of the probe. (*D*) Effect of pharmacological modulation of thyroid function on ICER expression in the thyroid gland. Western blot of thyroid gland extracts using an anti-CREM affinity-purified rabbit antiserum (18). Lane 1, euthyroid rat; lane 2, rat treated with methimazole (MMI; 0.05% in drinking water) for 8 days; lane 3, rat treated with T_3 (100 μg/100 g of body weight) for 8 days. Each sample is representative of a series of five animals. Bacterially produced CREMτ, -β, and -γ and ICER and ICERγ (lane 4) are shown as standards.

tion to hypophysectomized rats can induce ICER expression in their thyroid gland. Fig. 1*B* shows the kinetics of induction of ICER RNA after i.p. injection of 1 unit of bovine TSH per animal. In uninjected animals a low, but detectable, ICER level is present, which is dramatically increased after TSH injection, reaching a peak at 4 hr and sharply decreasing thereafter. Rapid metabolism of the injected hormone is likely to account for this kinetics of induction. The TSH effect on ICER expression was specific, since it could not be mimicked by two other pituitary hormones, prolactin and FSH (Fig. 1*C*). This is particularly significant, since the FSH α-subunit is also common to the TSH molecule.

Stimulation of the TSH-R by other means than its natural ligand can also lead to ICER inducibility in the hypophysectomized animal. Injection of serum from a patient with Graves disease, which contains a high level of TSH-R stimulating autoantibodies, resulted in ICER RNA induction (data not shown). To check the effects of altered endogenous TSH levels, we induced pharmacologically a state of hypo- or hyperthyroidism in otherwise healthy animals. Hypothyroid rats, which have high serum TSH after methimazole administration, expressed higher ICER levels than euthyroid control rats. In contrast, ICER levels were reduced in rats that were rendered hyperthyroid by injection of T_3 (Fig. 1*D*) and whose circulating TSH is very low. We have previously shown that stimulation of ICER expression is obtained through increased transcription from its intronic P_2 promoter, which contains several CREs (17). To our knowledge, this is the first example of a transcriptional repressor whose expression is directed by a pituitary hormone in the target gland. We have previously reported that FSH treatment can dramatically increase CREMτ activator expression in testis germ cells. In this latter case, however, induction is obtained through RNA stabilization by use of differential polyadenylylation sites (16).

The Kinetics of ICER Induction by TSH Correlates with TSH-R Down-Regulation. The rat thyroid FRTL-5 cell line is well characterized to be TSH-dependent for growth and expression of thyroid-specific markers, such as the TSH-R (11, 14). In this cell line, TSH treatment has been shown to result in the down-regulation of TRH-R mRNA at the transcriptional level. As early as 4 hr after TSH addition to the culture medium, TSH-R expression was repressed, after a transient up-regulation at earlier times (11). Importantly, the down-regulation of TSH-R has been demonstrated to require a newly synthesized factor, since it is abolished by cycloheximide (11).

Similar to what was observed in the animal (Fig. 1), TSH treatment of these cells also elicited the powerful induction of the ICER transcriptional repressor. Significantly, the elevation in ICER protein levels correlates with the concomitant decrease in TSH-R expression (Fig. 2*A*).

A striking temporal correlation also exists between TSH-mediated induction of ICER protein and down-regulation of TSH-R mRNA in the thyroid gland of the hypophysectomized rat (Fig. 2*B*). By RT-PCR, we were able to detect increased levels of TSH-R RNA levels in the hypophysectomized rat compared to the control animal (Fig. 2*B*). This finding is consistent with the reported increase in the number of TSH-Rs in the thyroid gland of hypophysectomized rats and increased response to TSH in terms of cAMP production, compared to control rats (25). By 1 hr after TSH administration, there was a transient increase in TSH-R RNA levels, which was rapidly followed by its down-regulation, reaching a nadir between 2 and 4 hr after TSH injection. Subsequently, a partial recovery in the levels of receptor transcripts occurred, but the levels were still lower than the baseline after 8 hr. The modulation of TSH-R RNA levels in the first hours after TSH administration to the hypophysectomized rat closely follows the kinetics observed *in vitro* in FRTL-5 cells (11). The kinetics of TSH-R RNA down-regulation after TSH administration closely mirrors the kinetics of ICER induction (Fig. 2*B*).

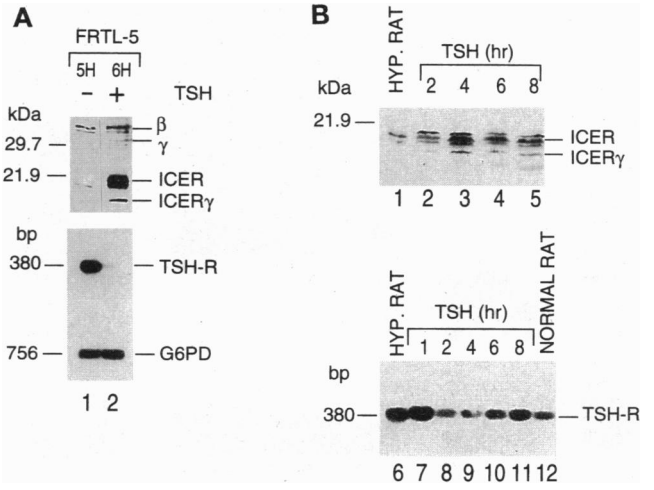

FIG. 2. Expression of the TSH-R is inversely correlated with ICER induction. (*A*) TSH induces ICER expression in FRTL-5 thyroid cells. FRTL-5 cells were cultured for 1 week in medium without TSH (5H; lane 1) or kept in the constant presence of TSH (6H; lane 2). Expression of CREM proteins and TSH-R and G6PD RNA are shown for the two different culture conditions. Migration of bacterially produced CREM proteins is shown on the right side of the Western blot. (*B*) Time course of ICER protein and TSH-R RNA expression *in vivo* in the hypophysectomized (HYP.) rat thyroid after i.p. injection of 1 unit of bovine TSH. ICER (lane 1) and TSH-R (lane 6) levels are shown in the hypophysectomized rat and 2, 4, 6, and 8 hr (ICER; lanes 2–5) or 1, 2, 4, 6, and 8 hr (TSH-R; lanes 7–11) after hormone administration. Normal rat TSH-R level is also shown (lane 12). Equivalent loadings of thyroid cDNA for TSH-R amplification were verified by G6PD RT-PCR.

Thus, increases in ICER levels correlate with a transcriptional down-regulation of the TSH-R. In this respect, it should be pointed out that the levels of TSH-R in the rat thyroid gland have been demonstrated to be down-regulated by propylthiouracil treatment, which induces hypothyroidism with high circulating TSH levels (26). Hypothyroidism induced by methimazole treatment, which shares with propylthiouracil the same pharmacological action on iodine organification and thyroid hormone production, is characterized by elevated ICER levels in the thyroid gland (Fig. 1*D*), constituting another example of inverse correlation between ICER and TSH-R expression.

A CRE in the TSH-R Promoter Is a Target for ICER. The TSH-R gene promoter contains a CRE (TGAGGTCA) between positions −139 and −132 (27). Reporter constructs harboring a minimal promoter containing this CRE are subject to negative regulation by forskolin in transfected thyroid cells (27, 28). Bacterially expressed ICER proteins bind a fragment containing the TSH-R minimal promoter, which includes the CRE sequence (Fig. 3, lanes 1 and 2). ICER proteins also bind an oligonucleotide including the TSH-R CRE sequence (Fig. 3, lanes 3 and 4). Importantly, nuclear extracts from FRTL-5 cells contain TSH-R/CRE binding activity, which generates a complex of mobility similar to ICER (lanes 5 and 6). In addition, this binding activity is induced by TSH in FRTL-5 cells [compare 5H (lane 5) and 6H (lane 6) in Fig. 3]. This binding is specific as shown by competition experiments (lanes 7–15). To test whether the TSH-inducible complex that binds the TSH-R CRE contains ICER, we studied the effect of CREM-specific antibodies (18). Addition of the anti-CREM antiserum to the binding reaction completely eliminated the complex formation. Conversely, a nonimmune rabbit serum had no effect on binding of the complex (lanes 16–19). Thus, down-regulation of TSH-R expression is coupled with a parallel increase in ICER repressor protein, which binds to the CRE sequence in the TSH-R promoter. Significantly, the

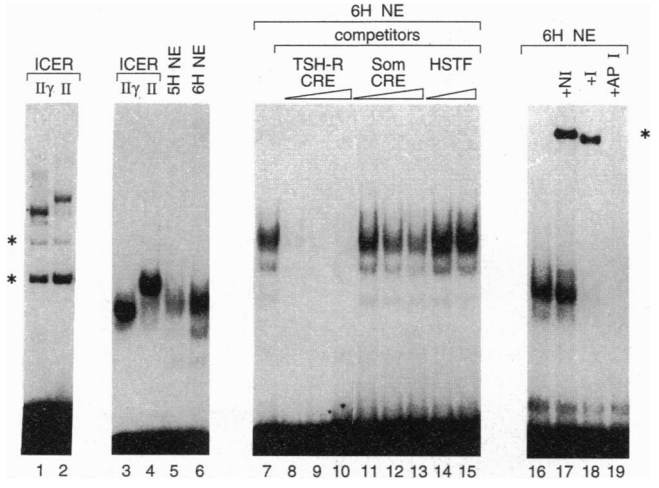

FIG. 3. FRTL-5 nuclear extracts (NE) form a TSH-inducible complex containing ICER with the CRE sequence in the TSH-R promoter. Bacterially produced ICERIIγ and ICERII proteins (1 μg) and 5 μg of nuclear extracts from FRTL-5 cells grown in the absence (5H) or in the presence (6H) of TSH were used in electrophoretic mobility shift assays. Lanes 1 and 2, binding of ICERIIγ (lane 1) and ICERII (lane 2) proteins to the −199/+1 TSH-R promoter sequence. Asterisks indicate nonspecific retarded complexes. Lanes 3–6, binding of ICERIIγ (lane 3), ICERII (lane 4), 5H extract (lane 5), and 6H extract (lane 6) to the TSH-R promoter −146/−114 sequence. Lanes 7–15, competition of 6H nuclear extract binding to the TSH-R −146/−114 sequence (lane 7), using 20, 60, and 100 ng of unlabeled −146/−114 oligonucleotide (lanes 8–10), 20, 60, and 100 ng of unlabeled somatostatin (Som) CRE oligonucleotide (lanes 11–13), and 20 and 60 ng of unlabeled heat shock transcription factor (HSTF) binding site oligonucleotide (lanes 14 and 15). An anti-CREM antiserum can interfere with 6H cell binding to the −146/−114 sequence. Binding of 6H nuclear extract (lane 16) is antagonized by 1 μl of an unpurified (I; lane 18) or abolished by an affinity-purified (AP I; lane 19) anti-CREM antiserum, while the same quantity of nonimmune serum (NI; lane 17) has no effect on binding. An asterisk indicates a nonspecific gel-retarded band present in samples when unpurified serum was used.

consensus somatostatin CRE only partially blocks the binding to the TSH-R CRE (lanes 11–13). This finding confirms that a specific code of ICER binding to various CRE sequences exists (unpublished results).

ICER Represses TSH-R Promoter Activity. To determine whether ICER can functionally repress gene expression driven by the TSH-R promoter, we performed transient transfection experiments in the human choriocarcinoma cell line JEG-3. We deliberately chose not to use FRTL-5 cells for transfection, considering their low transfection efficiency and the fact that TSH present in the culture medium, necessary to ensure cell viability after transfection, is able to powerfully down-regulate expression driven by the TSH-R promoter (27). Thus, we performed our experiments in JEG-3 cells, which are strongly responsive to stimulation of the cAMP signal-transduction pathway and are readily transfectable. In addition, these cells constitute a blank system to test TSH-R promoter activity, since they lack endogenous TSH-R expression and TSH dependence for growth. A reporter construct harboring the TSH-R minimal promoter cloned upstream from the luciferase gene (pGE-T200; ref. 22) showed increased activity as compared to basal levels in JEG-3 cells stimulated with forskolin or cotransfected with a PKA catalytic subunit expression vector (Fig. 4). This result is expected since in nonthyroid cells the TSH-R CRE acts as a constitutive and cAMP-inducible promoter element (28). Both basal and induced promoter activity were efficiently repressed by cotransfection with expression vectors containing ICER cDNAs (Fig. 4). Control reporters and expression vectors lacking the ICER coding

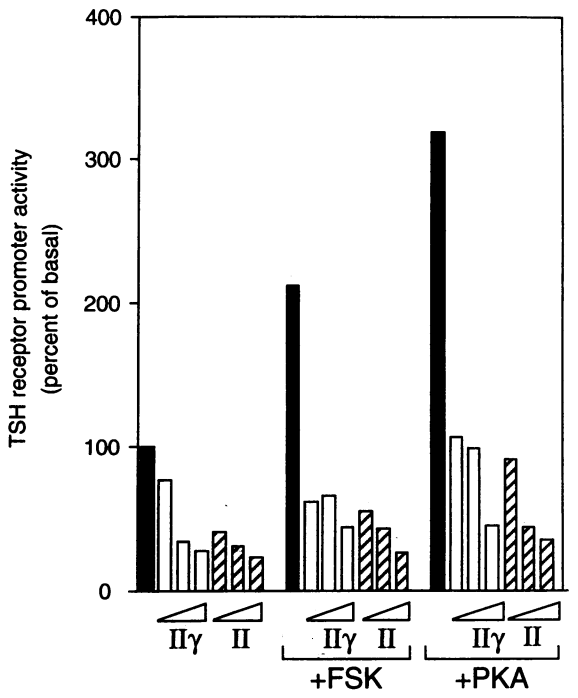

FIG. 4. ICER represses TSH-R expression. JEG-3 human choriocarcinoma cells were transiently transfected with pGE-T200 (22), which harbors the TSH-R minimal promoter cloned 5′ to the luciferase reporter gene. TSH-R promoter activity was assayed under basal conditions, in cells stimulated for 12 hr with forskolin (FSK; 10 μg/ml) and in cells cotransfected with 1 μg pCαEV, which encodes the PKA catalytic subunit. The effect of cotransfection with increasing doses (400 ng, 1 μg, and 2 μg) of pSV-ICERII (hatched bars) and pSV-ICERIIγ (open bars) expression vectors on basal and stimulated TSH-R promoter activity is shown. Cotransfection with the simian virus 40 expression vector without ICER had no effect (not shown). Results are expressed as a percentage of the basal activity, after normalization of luciferase activity to β-galactosidase activity. The data shown are representative of three independent experiments. Variability was <15%.

sequence confirmed the specificity of the results (data not shown).

DISCUSSION

The thyroid gland is entirely dependent on the pituitary hormone TSH for the regulation of thyroid hormone production (1). In addition, TSH is also a mitogen for thyroid cells, and increased TSH secretion is responsible for thyroid gland hypertrophia in pathological situations. This happens both in the case of pituitary adenomas secerning TSH, with a clinical situation of hyperthyroidism, and in the case of congenital defects in iodine incorporation into thyroid hormone or endemic goiter, which produce symptoms due to hypothyroidism (29).

The TSH-R has a large extracellular domain, which binds TSH, and seven hydrophobic transmembrane domains, which span the whole membrane and connect the ligand-binding domain to three intracytoplasmic loops (4). This intracellular domain is important for coupling the TSH-R to G proteins, which can activate both adenylate cyclase and phospholipase C, generating the second messengers cAMP and phosphatidylinositol bisphosphate (4). Binding of TSH to its own receptor reduces the cAMP response to a subsequent stimulation with the hormone; induction of refractoriness requires at least 30 min of incubation of the tissue with TSH (30, 31). This homologous desensitization has been shown to involve decreased coupling of the TSH-R with G_S (32) and is likely to

depend on receptor phosphorylation by a G protein-coupled receptor kinase and arrestin-like proteins (4, 33).

In addition to this rapid desensitization phenomenon, TSH-R mRNA expression has been shown to be down-regulated at the transcriptional level within a few hours after TSH stimulus (11, 34). This long-term modulation represents an important feedback mechanism to control cell response to TSH and subsequent cAMP production. An increase in intracellular cAMP levels, in fact, may trigger thyroid cell proliferation and thyroid hormone production, independently of the mechanisms of adenylate cyclase activation. This was demonstrated by studies where elevated cAMP production was caused by ectopic expression of the β_2-adrenergic receptor in FRTL-5 cells, which rendered the cells responsive to isoproterenol for growth and iodine uptake (35), or A_2-adenosine receptor in transgenic mice thyroid, with consequent gland hyperplasia and hyperthyroidism (36). In addition, mutations in the TSH-R gene have been found in some hyperfunctioning thyroid adenomas (12) and in a case of congenital hyperthyroidism (13); the mutated receptors were shown to be able to stimulate cAMP production in the absence of the ligand.

We have shown that one mechanism involved in the down-modulation of the TSH-R by its own ligand is represented by TSH induction of the transcriptional repressor ICER. CREM expression is induced in the hypophysectomized rat thyroid by TSH and other agonists for the TSH-R (human stimulating autoantibodies). The kinetics of ICER protein induction by TSH in the hypophysectomized rat correlates with the kinetics of TSH-R transcriptional down-regulation. Furthermore, ICER levels are very high in the thyroid gland of animals where hypothyroidism was induced by methimazole administration. In this situation, TSH receptor expression is diminished in the gland as compared to the euthyroid rat (26). ICER is also expressed at very high levels in the differentiated rat thyroid cell line FRTL-5 cultured in the presence of TSH, whereas cells deprived of TSH express very little ICER. TSH is known to powerfully repress expression of its own receptor at the transcriptional level in this cell line (11). ICER proteins can bind to the TSH-R promoter forming a complex that has a similar mobility to a TSH-inducible complex in FRTL-5 cells. In addition, ICER represses TSH-R promoter activity in transient transfection experiments. Thus, ICER appears to be a key player in the negative regulatory cascade triggered by TSH stimulation in thyroid cells. However, our results do not exclude the possibility that additional regulatory proteins could be involved in a sustained attenuation of TSH-R expression, maybe by cooperating with ICER itself.

In addition to CREM, other nuclear factors are involved in the fine regulation of the TSH-R promoter. TTF-1 has been recently implicated in thyroid-specific TSH-R gene expression and in its down-regulation by TSH in FRTL-5 cells (22, 37). It has been reported that the complex formed by TTF-1 with the TSH receptor promoter in a gel mobility assay is down-modulated by TSH with a kinetics similar to the TSH-R mRNA; in addition, TSH also reduces the levels of TTF-1 mRNA (37).

Triggering the expression of a transcriptional repressor through the cAMP pathway represents an effective mechanism to induce attenuation of gene expression after a first burst of activation for a pituitary hormone whose receptor is coupled to adenylate cyclase (5, 17). Considering the pivotal function of the cAMP-dependent signaling pathway in the response to pituitary hormones, it is likely that the phenomenon here described will be found to be general to produce homologous LTD of pituitary hormone receptors.

We thank N. S. Foulkes for critical reading of the manuscript; J. Stehle and P. Pévet for help; E. Borrelli, B. Bardoni, M. Lamas, K. Tamai, J. Lee, and F. Nantel for discussions; and D. Civitareale, E. Avvedimento, and G. S. McKnight for gift of material. The technical help of D. Bonn was greatly appreciated. We also thank F. Ruffenach, B. Boulay, and C. Werlé. E.L. is supported by a Fondation pour la Recherche Médicale fellowship. This work was supported by grants from Centre National de la Recherche Scientifique, Institut National de la Santé et pour la Recherche Médicale, Fondation de la Recherche Médicale, Centre Hospitalier Universitaire Régional, Association pour la Recherche contre le Cancer, and Rhône-Poulenc Rorer.

1. Utiger, R. D. (1987) in *Endocrinology and Metabolism*, eds. Felig, P., Baxter, J. D., Broadus, A. E. & Frohman, L. A. (MacGraw-Hill, New York), pp. 389–472.
2. Wong, C.-C., Döhler, K.-D., Atkinson, M. J., Geerlings, H., Hesch, R.-F. & von zur Mühlen, A. (1983) *Acta Endocrinol.* **102,** 377–385.
3. Akamizu, T., Ikuyama, S., Saji, M., Kosugi, S., Kozak, C., Wesley McBride, O. & Kohn, L. D. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5677–5681.
4. Nagayama, Y. & Rapoport, B. (1992) *Mol. Endocrinol.* **6,** 145–156.
5. Lalli, E. & Sassone-Corsi, P. (1994) *J. Biol. Chem.* **269,** 17359–17362.
6. Van Heuverswyn, B., Streydio, C., Brocas, H., Refetoff, S., Dumont, J. & Vassart, G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5941–5945.
7. Civitareale, D., Lonigro, R., Sinclair, A. J. & Di Lauro, R. (1989) *EMBO J.* **8,** 2537–2542.
8. Francis-Lang, H., Price, M., Polycarpou-Schwarz, M. & Di Lauro, R. (1992) *Mol. Cell. Biol.* **12,** 576–588.
9. Hansen, C., Javaux, F., Juvenal, G., Vassart, G. & Cristophe, D. (1989) *Biochem. Biophys. Res. Commun.* **160,** 722–731.
10. Avvedimento, E. V., Musti, A. M., Ueffing, M., Obici, S., Gallo, A., Sanchez, M., DeBrasi, D. & Gottesman, M. E. (1991) *Genes Dev.* **5,** 22–28.
11. Saji, M., Akamizu, T., Sanchez, M., Obici, S., Avvedimento, E., Gottesman, M. E. & Kohn, L. D. (1992) *Endocrinology* **130,** 520–533.
12. Parma, J., Duprez, L., Van Sande, J., Cochaux, P., Gervy, C., Mockel, J., Dumont, J. & Vassart, G. (1993) *Nature (London)* **365,** 649–651.
13. Kopp, P., van Sande, J., Parma, J., Duprez, L., Gerber, H., Joss, E., Jameson, J. L., Dumont, J. E. & Vassart, G. (1995) *N. Engl. J. Med.* **332,** 150–154.
14. Ambesi-Impiombato, F. S., Parks, L. A. M. & Coon, H. G. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3455–3459.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
16. Foulkes, N. S., Schlotter, F., Pévet, P. & Sassone-Corsi, P. (1993) *Nature (London)* **362,** 264–267.
17. Molina, C. A., Foulkes, N. S., Lalli, E. & Sassone-Corsi, P. (1993) *Cell* **75,** 875–886.
18. Delmas, V., van der Hoorn, F., Mellström, B., Jégou, B. & Sassone-Corsi, P. (1993) *Mol. Endocrinol.* **7,** 1502–1514.
19. Kawasaki, E. S. (1990) in *PCR Protocols*, eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Academic, San Diego), pp. 21–27.
20. Foulkes, N. S., Borrelli, E. & Sassone-Corsi, P. (1991) *Cell* **64,** 739–749.
21. Andrews, N. C. & Faller, D. V. (1991) *Nucleic Acids Res.* **19,** 2499.
22. Civitareale, D., Castelli, M. P., Falasca, P. & Saiardi, A. (1993) *Mol. Endocrinol.* **7,** 1589–1595.
23. deWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7,** 725–737.
24. Stehle, J. H., Foulkes, N. S., Molina, C. A., Simonneaux, V., Pévet, P. & Sassone-Corsi, P. (1993) *Nature (London)* **365,** 314–320.
25. Holmes, S. D. & Field, J. B. (1980) *Endocr. Res. Commun.* **7,** 205–219.
26. Witte, A., Henderson, B., Zakarija, M. & McKenzie, J. M. (1980) *Endocrinology* **106,** 1489–1497.
27. Ikuyama, S., Niller, H. H., Shimura, H., Akamizu, T. & Kohn, L. D. (1992) *Mol. Endocrinol.* **6,** 793–804.
28. Ikuyama, S., Shimura, H., Hoeffler, J. P. & Kohn, L. D. (1992b) *Mol. Endocrinol.* **6,** 1701–1715.
29. Ingbar, S. H. (1987) in *Harrison's Principles of Internal Medicine*, eds. Braunwald, E., Isselbacher, K. J., Petersdorf, R. G., Wilson, J. D. Martin, J. B. & Fauci, A. S. (McGraw-Hill, New York), pp. 1732–1752.
30. Rapoport, B. & Adams, R. J. (1976) *J. Biol. Chem.* **251,** 6653–6661.
31. Shuman, S. J., Zor, U., Chayoth, R. & Field, J. B. (1976) *J. Clin. Invest.* **57,** 1132–1141.
32. Rapoport, B., Filetti, S., Takai, N. & Seto, P. (1982) *FEBS Lett.* **146,** 23–27.
33. Lefkowitz, R. J., Cotecchia, S., Kjelsberg, M. A., Pitcher, J., Koch, W. J., Inglese, J. & Caron, M. G. (1993) *Adv. Second Messenger Phosphoprotein Res.* **28,** 1–9.
34. Foti, D., Catalfamo, R., Russo, D., Costante, G. & Filetti, S. (1991) *J. Endocrinol. Invest.* **14,** 213–218.
35. Hen, R., Axel, R. & Obici, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4785–4788.
36. Ledent, C., Dumont, J. E., Vassart, G. & Parmentier, M. (1992) *EMBO J.* **11,** 537–542.
37. Shimura, H., Okajima, F., Ikuyama, S., Shimura, Y., Kimura, S., Saji, M. & Kohn, L. D. (1994) *Mol. Endocrinol.* **8,** 1049–1069.