

Handbook of Experimental Pharmacology

Volume 86

Editorial Board

G. V. R. Born, London

P. Cuatrecasas, Research Triangle Park, NC

H. Herken, Berlin

A. Schwartz, Cincinnati, OH

The Cholinergic Synapse

Contributors

D. V. Agoston · T. Bartfai · E. Borroni · A. N. Davison · I. Ducis
F. Fonnum · E. Giacobini · I. Hanin · J. Järv · D. A. Johnson
H. Kilbinger · W.-D. Krenz · M. E. Kriebel · K. Krnjević · A. Maelicke
J. Massoulié · E. G. McGeer · P. L. McGeer · G. R. Pilar · P. E. Potter
G. P. Richardson · P. J. Richardson · D. W. Schmid · H. Stadler
J.-P. Toutant · K. V. Toyka · S. Tuček · M. Tytell · K. Wächtler
V. P. Whittaker · H. Zimmermann

Editor

V. P. Whittaker



Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

Contents

Part I. Model Cholinergic Systems

CHAPTER 1

Model Cholinergic Systems: An Overview

V.P. WHITTAKER. With 2 Figures	3
A. The Value of Model Systems	3
B. Pros and Cons of Selected Model Systems	3
I. Cephalopod Optic Lobe	3
II. Electric Organs	4
III. Myenteric Plexus-Longitudinal Muscle Preparation	5
IV. Ganglion Preparations	6
V. Mammalian Central Nervous System Preparations	8
VI. Amphibian and Mammalian Voluntary Muscle	10
References	18

CHAPTER 2

Model Cholinergic Systems: The Electromotor System of *Torpedo*

V.P. WHITTAKER and D. W. SCHMID. With 7 Figures	23
A. Embryology	23
B. Components of the Electromotor System	26
I. Electric Lobe	26
II. Axons	31
III. Electric Organ	32
C. Conclusions	36
References	36

CHAPTER 3

Model Cholinergic Systems: The Avian Ciliary Ganglion

G.R. PILAR and D.A. JOHNSON. With 2 Figures	41
A. Introduction	41
B. Anatomy	41

C. Electrophysiology and Biochemistry	45
I. Chemical and Electrical Components in Synaptic Transmission ..	45
II. Presence of Neuropeptides and its Implications	47
III. Presence of Presynaptic ACh Receptors	48
IV. Postsynaptic Receptor Heterogeneity	49
V. Regulation of ACh Synthesis	50
D. Cell and Tissue Culture	51
E. Concluding Remarks	52
References	52

Part II. Biology of the Cholinergic Synapse

CHAPTER 4

Phylogeny of the Cholinergic Synapse

K. WÄCHTLER. With 8 Figures	57
A. Introduction	57
B. Variation at the Synaptic Level	59
I. Parameters Considered	59
II. Synthesis of ACh	60
III. Termination of the Action of ACh	60
IV. Characteristics of ACh Receptors	61
C. Qualitative and Quantitative Changes in Cholinergic Innervation	63
I. Qualitative Changes	63
II. Quantitative Changes	65
D. Conclusions	73
References	74

CHAPTER 5

Development of the Cholinergic Synapse: Role of Trophic Factors

G. P. RICHARDSON	81
A. Introduction	81
B. Parasympathetic Ciliary Neurons	81
I. Ciliary Ganglion as a Model System	81
II. Necessity of Trophic Factors for the Survival and Growth of Ciliary Neurons <i>In Vitro</i>	82
III. Developmental Regulation of Survival Activity for Ciliary Neurons	83
IV. Characterization of Ciliary Neuron Survival Factors	83
V. Factors Affecting the Growth and Development of Ciliary Neurons <i>In Vitro</i>	84
VI. Factors Stimulating Neurite Growth in Ciliary Neuron Cultures ..	85
C. Spinal Cord Neurons	87

I. Greater Complexity of the System	87
II. Survival Factors for Spinal Cord Neurons	87
III. Factors Stimulating ChAT Activity in Spinal Cord Cultures	88
IV. Factors Stimulating Neurite Growth in Spinal Cord Cultures	91
D. Sympathetic Neurons	92
I. Evidence for a Cholinergic Subpopulation	92
II. Induction of Cholinergic Transmitter Status in Sympathetic Neurons	92
III. Characterization of the Cholinergic-Specifying Factor	93
E. Conclusions	94
References	95

Part III. Molecular Components of the Cholinergic System

CHAPTER 6

Estimation of Acetylcholine and Choline and Analysis of Acetylcholine Turnover Rates *In Vivo*

P. E. POTTER and I. HANIN	103
A. Introduction	103
B. Preparation of Samples for ACh and Choline Analysis	104
I. General Requirements for Measurement of ACh and Choline ...	104
II. Extraction of ACh and Choline from Tissues, Subcellular Fractions and Perfusates	104
III. Preservation of ACh and Choline <i>Post-Mortem</i>	104
C. Analysis of ACh and Choline	105
I. Bioassay	105
II. Chemical Techniques Before 1972, Excluding Radioenzymatic and Gas Chromatographic (GC) Assays	107
III. Radioenzymatic Assays	107
IV. GC and GCMS Analyses	108
V. New Developments Since 1978	109
D. Methods for Measuring ACh Turnover	110
I. General Considerations	110
II. Non-isotopic Methods	111
III. Isotopic Methods	113
E. Concluding Remarks	117
References	118

CHAPTER 7

Choline Acetyltransferase and the Synthesis of Acetylcholine

S. TUČEK. With 4 Figures	125
A. Introduction	125
B. Choline Acetyltransferase	126

I. The Enzyme Molecule	126
II. Catalytic Action	129
III. Cellular and Subcellular Localization	133
C. Supply of Substrates	136
I. Acetylcoenzyme A	136
II. Choline	139
III. Source of Choline for ACh Synthesis	144
D. Regulation of ACh Synthesis	146
I. Kinetic Considerations	146
II. Regulatory Factors	147
References	151

CHAPTER 8a

Vertebrate Cholinesterases: Structure and Types of Interaction

J. MASSOULIÉ and J.-P. TOUTANT. With 8 Figures	167
A. Introduction	167
B. Duality of ChEs: AChE and BuChE	168
C. Molecular Forms of ChEs: The Value and Limitations of a General Scheme and a Nomenclature for Their Quaternary Structure	170
D. Electric Organs of Fish: A Privileged Source of AChE for Biochemical Studies	172
E. Quaternary Structure and Interactions of Asymmetric Forms	173
I. <i>Electrophorus</i>	173
II. <i>Torpedo</i>	175
III. Asymmetric AChE Forms from Other Species, BuChE Asymmetric Forms, Hybrid AChE-BuChE Asymmetric Forms ..	177
IV. Ionic Interactions	178
V. Asymmetric AChE Forms and the Basal Lamina	181
F. Physicochemical Properties of Globular Forms	182
I. Solubility and Hydrophobicity: Operational Definition of Amphiphilic Molecules	182
II. Solubility of ChE Globular Forms: Soluble and Detergent-Soluble Fractions	182
III. Non-amphiphilic or Hydrophilic Globular Forms	183
IV. Membrane-Bound, Intracellular and Extracellular Amphiphilic Forms	184
V. Interactions with Detergent Micelles	185
VI. Dimeric Forms Containing a Hydrophobic Glycolipid Anchor ..	187
VII. Structure of G ₄ Forms Containing a Distinct Hydrophobic Subunit	191
VIII. Solubilization Artefacts, Modifications of the Solubilized Forms	192
IX. Catalytic Activity and Hydrophobic Interactions	193
X. Thermal Stability	193
G. Variability of the Catalytic Subunits	194

I. Catalytic Subunits of AChE and BuChE in Vertebrates: Analysis by SDS-PAGE	194
II. Specificity of Inhibitory Antibodies and Peptide Toxins	197
III. Comparison Between Catalytic Subunits of Different AChE Fractions Within a Given Species	197
IV. Heterogeneity of AChE Globular Forms in Non-denaturing Electrophoresis	199
V. Binding of Lectins and Heterogeneity of Glycosylation of AChE Molecules	199
H. Immunochemical Studies	200
I. Immunochemical Cross-Reactivity Between AChE and BuChE ..	200
II. Interspecific Cross-Reactivity	200
III. Immunochemical Differences Between AChE Fractions Within a Given Organism	201
I. Molecular-Biological Studies	203
I. Characterization of AChE mRNAs and Their Translation Products	203
II. Characterization of AChE cDNA Clones	204
III. Primary Structures of ChEs	205
J. Conclusions	210
References	211

CHAPTER 8b

Cholinesterases: Tissue and Cellular Distribution of Molecular Forms and Their Physiological Regulation

J.-P. TOUTANT and J. MASSOULIÉ. With 1 Figure	225
A. Introduction	225
B. Tissue Distribution of ChE Molecular Forms	225
I. General Considerations	225
II. Distribution of AChE Molecular Forms at Neuromuscular Junctions	226
III. Ganglionic Synapses of the Autonomic Nervous System	228
C. Subcellular Distribution of AChE Molecular Forms in Muscle and Nerve Cells	228
I. Determination of Endo- and Ectocellular Enzyme Activities	228
II. Distribution of Molecular Forms in Ecto- and Endocellular Compartments	229
D. Biosynthesis and Renewal of AChE Molecular Forms	231
I. <i>In Vitro</i> Studies	231
II. Nervous Tissue and Muscle <i>In Vivo</i>	233
E. Intracellular Transport and Secretion of AChE Molecular Forms	234
I. Intracellular Transport	234
II. Secretion	235
F. Regulation of the Synthesis of AChE by Muscle and Nerve Cells During Development <i>In Vivo</i> and <i>In Vitro</i>	236

I. Evolution of AChE Activity and Molecular Forms in Muscle and Nerve Cells During Embryogenesis	236
II. Relationship Between Synthesis of A Forms and Innervation of Muscle and Nerve Cells <i>In Vivo</i>	238
III. Regulation of the Synthesis of A Forms by Muscle and Nerve Cells <i>In Vitro</i>	239
IV. Localization of AChE on Muscle Membranes	242
G. Non-cholinergic Functions of ChEs	243
I. Adult Tissues: New Substrates for ChEs	243
II. "Embryonic ChE": A Cholinergic Function in the Absence of Innervation?	245
H. Effects of Peptides on ChEs	246
I. Effect on the Activity of AChE	246
II. Effect on the Synthesis of ChE	246
I. ChEs in Pathological States and Their Evolution with Aging	247
I. Muscular Dystrophy	247
II. Human Pathology	248
III. AChE and the Prenatal Diagnosis of Neural Tube Defects	250
IV. Aging	250
J. Conclusion	251
References	252

CHAPTER 9

Structure and Function of the Nicotinic Acetylcholine Receptor

A. MAELICKE. With 4 Figures	267
A. Introduction and Scope	267
B. Structure of the nAChR	268
I. Primary Structure	268
II. Secondary Structure and Topography	270
III. Tertiary Structure	271
IV. Quaternary Structure	273
V. Functional Domains	275
VI. Conformations	279
VII. Classification of ACh Receptors According to Structure	279
C. Molecular Genetics	281
I. Structure of nAChR Genes	281
II. nAChR Gene Expression and Processing	283
D. Function of the nAChR	284
I. Ligand Binding	284
II. Channel Gating	287
III. Modulation of Binding and Response	289
IV. Molecular Mechanism of Function	290
References	293

CHAPTER 10

Muscarinic Acetylcholine Receptors

J. JÄRV and T. BARTEAL. With 2 Figures	315
A. Introduction	315
I. Two Main Types of ACh Receptor	315
II. Biochemistry of mAChRs	315
III. Localization of mAChRs	317
B. Assays of Muscarinic Receptors and Muscarinic Ligands	320
I. Binding Studies	320
II. Assay of Muscarinic Agonist and Antagonist Actions	324
III. Muscarinic Agonist Effects in Intact Animals	325
C. Clinical Application of Muscarinic Drugs	327
I. Therapeutic Uses of Muscarinic Drugs	327
II. Acute Poisoning with Muscarinic Agonists	327
III. Acute Poisoning with Muscarinic Antagonists	327
References	338

Part IV. Cellular Organization of the Cholinergic System

CHAPTER 11

Cholinergic Synaptic Vesicles

H. ZIMMERMANN. With 2 Figures	349
A. Introduction	349
B. Isolation of Cholinergic Synaptic Vesicles	349
C. General Properties	350
D. Vesicle Core	352
I. Constituents of the Core	352
II. ACh and ATP	352
III. Metal Ions	353
IV. pH and Membrane Potential	353
V. Proteoglycan	354
VI. Peptides	355
E. Vesicle Membrane	355
I. Composition	355
II. Membrane Lipids	355
III. Polypeptide Composition	357
IV. Vesicular Proteins	357
F. Vesicular Transport Systems	363
I. Types of System Present	363
II. Vesicular Proton Pump	363
III. Vesicular Uptake of Ca^{2+}	365
IV. Vesicular Uptake of Opioid Peptides	365
V. Vesicular Uptake of ATP	365

VI. Vesicular Uptake of ACh	366
G. Dynamic Properties of Synaptic Vesicles	368
I. Morphological Alterations on Nerve Stimulation	368
II. Biochemical Alterations on Nerve Stimulation	370
III. Mechanism of Exocytosis	373
IV. Gated Release of ACh	374
V. Life-Cycle of the Synaptic Vesicle	374
References	375

CHAPTER 12

Isolation of Cholinergic Nerve Terminals

P.J. RICHARDSON. With 3 Figures	383
A. Introduction	383
B. Historical Aspects	383
C. Heterogeneous Mammalian Nerve-Terminal Preparations	383
D. Heterogeneous Non-mammalian Nerve Terminals	385
E. Purely Cholinergic Nerve-Terminal Preparations from Electric Organs	386
I. Types of Preparation Available	386
II. Purity and Functional State of Electromotor Synaptosome Preparations	387
III. Presynaptic Plasma Membranes	388
F. Affinity Purification of Mammalian Cholinergic Nerve Terminals	389
I. Separation Procedure	389
II. Characteristics of the Preparation	391
G. Summary	392
References	394

CHAPTER 13

Axonal Transport in Cholinergic Neurons

M. TYTELL and H. STADLER	399
A. Introduction	399
B. General Protein Transport in Cholinergic Nerves	399
C. Transport of Distinctive Components of the Cholinergic Neuron	400
I. Acetylcholine	400
II. Acetylcholinesterase	401
III. Choline Acetyltransferase	403
IV. Cholinergic Synaptic Vesicle-Associated Proteins	403
D. Interpretation of the Transport of Cholinergic-Specific Substances in the Context of General Axonal Protein Transport	403
References	405

CHAPTER 14

The High-Affinity Choline Uptake System

I. DUCIS	409
A. Introduction	409
B. Characteristics of HACU	410
I. Tissue and Site Specificity	410
II. Physiological Significance	411
III. Structure-Activity Relationship Studies	415
IV. Bioenergetics of Uptake	416
V. Possible Role of Sialocompounds, Lipids and Steroids	423
C. Identification of the HACU System	425
I. Criteria for Identification	425
II. Attempted Identification of Presynaptic Plasma Membrane Components and Their Modification	426
III. Binding of Hemicholinium-3	426
IV. Labelling with Covalent Probes	428
V. Reconstitution of Choline Uptake Activity	429
D. Areas for Future Investigation	431
I. Functional Ligands and Components	431
II. Arrangement of Transport System in the Membrane	432
III. Isolation of Transport System	433
IV. Possible Role of Lipids	433
V. Conformational Changes of the Transporter Associated with Choline Uptake	436
References	437

CHAPTER 15

Cholinergic-Specific Antigens

V.P. WHITTAKER and E. BORRONI. With 7 Figures	447
A. Introduction	447
I. General Strategy	447
II. The Problem of Unwanted Antibodies	449
III. Complement Lysis Test	449
B. Surface Antigens Specific for Cholinergic Nerve Terminals	450
I. Identification of Cholinergic-Specific Gangliosides	450
II. Applications of Anti-Chol-1 Antisera	451
III. Possible Functional Significance of Chol-1	455
IV. Complement-Mediated Lysis of Synaptosomes Induced by Anti-ChAT Antisera	457
C. Synaptic Vesicle Proteoglycans	457
I. From <i>Torpedo marmorata</i>	457
II. From Other Torpedine Fish	459
References	461

CHAPTER 16

Cholinergic False Transmitters

V.P. WHITTAKER. With 3 Figures	465
A. Definition and Structural Requirements	465
I. Definition	465
II. Adrenergic System	465
III. Cholinergic System	466
B. Differential Labelling of Transmitter Pools by False Transmitters	468
I. Electric Organ	468
II. Mammalian Preparations	473
C. Conclusions	475
References	475

CHAPTER 17

Cholinergic Co-transmitters

D.V. AGOSTON. With 2 Figures	479
A. Introduction	479
I. Scope of the Chapter: ATP and Neuropeptides	479
II. Terminology	480
III. Criteria to Be Satisfied	481
B. ATP as a Neuroactive Compound	482
I. ATP and Its Derivatives in Neuronal Function	482
II. ATP in Chromaffin Granules	483
III. ATP in the Cholinergic System of <i>Torpedo</i>	484
IV. ATP in Mammalian Cholinergic Systems	486
V. The Concept of Independent Purinergic Terminals	488
VI. Must ATP Be Reckoned as a Cholinergic Co-transmitter?	489
C. Evidence for Co-localization of Certain Neuropeptides and ACh	491
I. Discovery of Neuropeptides	491
II. Discovery of Co-localization	492
III. Histochemical and Cytochemical Evidence for Co-localization ...	498
IV. Cell-Biological and Biochemical Evidence	502
V. Physiological and Pharmacological Evidence	506
VI. Consequences of Coexistence of ACh and Neuropeptides	517
References	523

Part V. Peripheral Cholinergic Synapses

CHAPTER 18

The Neuromuscular Junction

M.E. KRIEBEL. With 15 Figures	537
A. Introduction	537
B. Quantal Basis of Transmitter Release	540

C. Classes of Quanta	543
I. Bell-mEPPs and Skew- (sub-)mEPPS	543
II. Schwann-Cell mEPPs	550
III. Giant mEPPs and Doublet mEPPs	552
IV. Slow Skew-mEPPs	553
D. Subunit Hypothesis of the Quantum of Transmitter Release	555
E. Morphological Correlates of Bell-mEPPs and Skew-mEPPs	560
F. Concluding Remarks	561
References	562

CHAPTER 19

The Electromotor Synapse

W.-D. KRENZ. With 4 Figures	567
A. Introduction	567
B. Gross Physiology	567
C. Physiology of Single Electrocytes	568
D. Microphysiology of Transmission at the EMJ	571
I. Extracellular Electroplaque Currents	571
II. Intracellular Electroplaque Potentials	573
III. Quantal Analysis of Electroplaque Potentials	575
E. Conclusions	576
References	577

CHAPTER 20

The Autonomic Cholinergic Neuroeffector Junction

H. KILBINGER	581
A. Introduction	581
B. Release of ACh	581
I. Resting Release	582
II. Electrically Evoked Release	582
III. Release of Vasoactive Intestinal Peptide as a Co-transmitter in Cholinergic Nerves	583
C. Modulation of ACh Release by Muscarinic Autoreceptors	584
I. Prejunctional Autoreceptors	584
II. Soma-Dendritic Autoreceptors	589
D. Effects of ACh on Effector Organs: Mediation Through Subtypes of mAChRs	591
References	591

Part VI. Central Cholinergic Systems**CHAPTER 21****Central Cholinergic Pathways: The Biochemical Evidence**

F. FONNUM	599
A. Introduction	599
B. Biochemical Markers	599
C. Preparation of Tissue	600
I. Single Cell Preparations	600
II. Microdissection from Freeze-Dried Sections	601
III. Micropunches from Frozen Sections	601
IV. Micropunches from Fresh Tissue	601
V. Lesions	601
D. Distribution of Cholinergic Markers in the Brain	602
E. Localization of Cholinergic Pathways in the Brain	603
I. Olfactory Bulb	603
II. Cerebral Cortex	604
III. Hippocampal Region	604
IV. Amygdaloid Complex	605
V. Striatum	605
VI. Thalamus	606
VII. Habenulo-Interpeduncular System	606
VIII. Hypothalamus	607
IX. Cerebellum	607
X. Brain Stem	608
XI. Spinal Cord	608
XII. Retina	608
References	609

CHAPTER 22**Central Cholinergic Pathways: The Histochemical Evidence**

E. G. MCGEER and P. L. MCGEER. With 2 Figures	615
A. Introduction	615
B. Techniques	615
I. Immunohistochemistry for ChAT	615
II. AChE Histochemistry	618
III. Ancillary Techniques	618
C. Central Cholinergic Systems	622
I. Cholinergic Cell Groups	622
II. Cholinergic Pathways	624
D. Conclusions	626
References	627

CHAPTER 23

Central Cholinergic Transmission: The Physiological Evidence

K. KRNJEVIĆ. With 12 Figures	633
A. Introduction	633
B. Nicotinic Synapses	634
I. Characteristics of Peripheral Synapses	634
II. Central Nicotinic Synapses	634
C. Muscarinic Synapses	643
I. General Considerations	643
II. Forebrain Cholinergic System	644
D. Conclusions	651
References	655

Part VII. Neuropathology of Cholinergic Transmission

CHAPTER 24

The Cholinergic System in Aging

E. GIACOBINI. With 6 Figures	665
A. The Process of Aging of Cholinergic Synapses: Hypotheses and Facts	665
B. Aging of the Neuromuscular Junction	665
I. Skeletal Muscle of Rodents	665
II. Avian Iris NMJ	670
C. ACh Synthesis and Metabolism in Brains of Aging Animals	677
D. Cholinergic Receptors and Their Pharmacology	680
E. Experimental Models of Dementia and Aging Based on Chemical Lesions of Cholinergic Systems	681
F. Brain Transplants into ACh-Deficient Animals	686
G. Cholinergic Deficits in Normal Aging and in Alzheimer's Disease ...	687
References	689

CHAPTER 25

Disorders of Cholinergic Synapses in the Peripheral Nervous System

K. V. TOYKA	697
A. Introduction	697
B. Presynaptic Disorders of the Neuromuscular Junction	697
I. Lambert-Eaton Myasthenic Syndrome	697
II. Intoxication with Botulinum Toxin in Man	702
III. Other Toxins Acting on Presynaptic Terminals	702
IV. Congenital Myasthenia	703
C. Postsynaptic Disorders of the Neuromuscular Junction	704
I. Myasthenia Gravis (Acquired Autoimmune Myasthenia)	704

II. Congenital Myasthenia	710
III. Inhibition of AChE	711
IV. Snake Neurotoxins	712
D. Disorders of the Peripheral Somatic Nervous System	712
I. Neuropathies with Predominant Motor Fibre Dysfunction and Motoneuron Disorders	712
II. Amyotrophic Lateral Sclerosis	714
E. Autonomic Neuropathies	715
I. Clinical Tests for Autonomic Involvement	715
II. Cholinergic Autonomic Dysfunction in Diabetes Mellitus	715
III. Acute Pandysautonomia	717
IV. Animal Models of Autonomic Neuropathy	718
V. Generalized Smooth Muscle Disease with Intestinal Pseudoobstruction	718
F. Glossary	719
References	719

CHAPTER 26

Central Cholinergic Neuropathologies

A. N. DAVISON. With 4 Figures	725
A. Introduction	725
B. Pathological Processes	726
I. Disposing Factors	726
II. Metabolic	727
III. Genetic	727
IV. Neuronal Loss of Unknown Cause	727
V. Environmental Poisons and Infective Agents	728
C. Central Cholinergic Neuropathies	729
I. Alzheimer's Disease	729
II. Down's Syndrome	736
III. Parkinson's Disease	737
IV. Huntington's Chorea	737
V. Motoneuron Disease	738
D. Conclusion	739
References	739

Subject Index	745
----------------------------	-----