

Handbook of
Neurochemistry

SECOND EDITION

Volume 2
**EXPERIMENTAL
NEUROCHEMISTRY**

Edited by
Abel Lajtha

59.5934073
H236(2)
:2

Handbook of Neurochemistry

SECOND EDITION

Volume 2 EXPERIMENTAL NEUROCHEMISTRY

Edited by

Abel Lajtha

*Center for Neurochemistry
Ward's Island, New York*



PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Handbook of neurochemistry.

Includes bibliographical references and index.

Contents: v. 1. Chemical and cellular architecture--v. 2. Experimental neurochemistry.

1. Neurochemistry--Handbooks, manuals, etc. I. Lajtha, Abel. [DNLM: 1. Neurochemistry. WL 104 H434]

QP356.3.H36 1982

612/.814

82-493

ISBN 0-306-40972-0 (v. 2)

© 1982 Plenum Press, New York
A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

Contributors

K. Adriaenssens, Provincial Instituut voor Hygiëne, Antwerp, Belgium

Glen B. Baker, Neurochemical Research Unit, Department of Psychiatry, University of Alberta, Edmonton, Alberta T6G 2N8, Canada

Nicole Baumann, INSERM and CNRS Laboratory of Neurochemistry, Salpetriere Hospital, Paris 13, France

E. D. Bird, McLean Hospital, Harvard Medical School, Belmont, Massachusetts 02178

Alan A. Boulton, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan S7N OXO, Canada

Jonathan D. Brodie, Department of Psychiatry, New York University School of Medicine, New York, New York 10016

N. Chamoles, Laboratorio de Neuroquímica, Clínica del Sol, Buenos Aires, Argentina

Jørgen Clausen, Neurochemical Institute, Copenhagen, Denmark, and Institute of Biology and Chemistry, University of Roskilde, DK 4000 Roskilde, Denmark

Thomas B. Cooper, Analytical Psychopharmacology Laboratory, Rockland Research Institute, Orangeburg, New York 10962

Ronald T. Coutts, Neurochemical Research Unit, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada

David A. Durden, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan S7N OXO, Canada

David D. Gilboe, Departments of Neurosurgery and Physiology, University of Wisconsin Center for Health Sciences, Madison, Wisconsin 53706

- Fritz A. Henn*, University of Iowa, Department of Psychiatry, College of Medicine, Iowa City, Iowa 52242. Present address: Long Island Research Institute, State University of New York, Stony Brook, New York 11794.
- Suella W. Henn*, University of Iowa, Department of Psychiatry, College of Medicine, Iowa City, Iowa 52242. Present address: Long Island Research Institute, State University of New York, Stony Brook, New York 11794.
- R. Humbel*, Centre Hospitalier de Luxembourg, Luxembourg
- L. L. Iversen*, Neurochemical Pharmacology Unit, Medical Research Council Centre, Medical School, Cambridge CB2 2QH, England
- G. Jean Kant*, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012
- Barry B. Kaplan*, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York 10021
- Katrina L. Kelner*, Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030
- Francois Lachapelle*, INSERM and CNRS Laboratory of Neurochemistry, Salpêtrière Hospital, Paris, France
- Robert H. Lenox*, Neuroscience Research Unit, Department of Psychiatry, University of Vermont, Burlington, Vermont 05405
- A. Lowenthal*, Laboratory of Neurochemistry, Born Bunge Foundation, Universitaire Instelling Antwerpen, Antwerp, Belgium
- James L. Meyerhoff*, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012
- Volker Neuhoff*, Forschungsstelle Neurochemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany
- Jose M. Palacios*, Department of Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205. Present address: Sandoz Ltd., Preclinical Research, CH-4002 Basel, Switzerland.
- Ernest J. Peck, Jr.*, Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030
- Stephen R. Philips*, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan S7N 0X0, Canada
- John Rotrosen*, Psychiatry Service, Veterans Administration Medical Center, New York, New York 10010
- Stanley Stein*, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

N. M. van Gelder, Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Nora Volkow, Department of Psychiatry, New York University School of Medicine, New York, New York 10016

James K. Wamsley, Departments of Psychiatry and Anatomy, University of Utah, Salt Lake City, Utah 84132

David L. Wilson, Department of Physiology and Biophysics, University of Miami, School of Medicine, Miami, Florida 33101

Preface

The second volume of the *Handbook* does not parallel any volume of the first edition; it is one more sign, or reflection, of the expansion of the field. By emphasizing the experimental approach, it illustrates the tools that have recently become available for investigating the nervous system. Also, perhaps even more than other volumes, it illustrates the multidisciplinary nature of the field, requiring multidisciplinary methodology. It is now recognized that the availability of methodology is often the rate-limiting determinant of studies and that improvements or innovations in instrumentation can open up new avenues. A new improved method, although opening up new possibilities and being crucial to making advances, is only a tool whose use will determine its usefulness. If we do not recognize its possibilities, its use will be limited; if we do not recognize its limitations, it will mislead us. It is the possibilities and limitations and the results obtained that are illustrated here.

As with the other volumes of this *Handbook*, many more chapters could be included, and each of the present chapters could have been expanded severalfold. Some topics that could be included in this volume will be dealt with in later chapters; some are better discussed in texts of other disciplines. The purpose was not to include so much detail that further literature searches would not be necessary—it was to evaluate the approaches, the limitations, the possibilities, and then to indicate where further details can be found. The fact that the details of most of the approaches described here were not available when the first edition was written is but another illustration of the rapid and exciting development of neurochemistry.

Abel Lajtha

Contents

Chapter 1

RNA-DNA Hybridization: Analysis of Gene Expression

Barry B. Kaplan

1. Introduction	1
2. Principles of RNA-Driven Hybridization Reactions	2
3. Variables Affecting Hybridization	3
3.1. Temperature	4
3.2. Ionic Strength and pH	4
3.3. Nucleic Acid Fragment Length	4
3.4. Viscosity	5
4. Specificity of Hybridization	5
5. Use of Organic Reagents	5
6. Hybridization Assays	6
6.1. Hydroxyapatite Chromatography	6
6.2. S_1 Nuclease Assay	7
6.3. Comparison of Methods	7
7. Hybridization Probes	8
7.1. Saturation Hybridization	8
7.2. Complementary DNA Analysis	10
7.3. Comparison of Experimental Approaches	12
8. Complexity of Gene Expression in Brain	14
8.1. Transcriptional Level	14
8.2. Translational Level	16
9. Cell and Regional Distribution of Gene Transcripts	18
9.1. Cell Lines of Neuroectodermal Origin	18
9.2. Major Brain Regions	18
10. Gene Expression during the Animal Life-Span	19
10.1. Postnatal Development	20
10.2. Aging Studies	20
11. Concluding Remarks	21
References	22

Chapter 2

Receptor Mapping by Histochemistry

James K. Wamsley and Jose M. Palacios

1. Introduction	27
1.1. Necessity of Localizing Receptors	27
1.2. Historical Background	28
1.3. Recent Developments in Receptor Autoradiography	30
2. Techniques of Localizing Neurotransmitter Receptors by Autoradiography	32
2.1. <i>In Vivo</i> Labeling and Processing	32
2.2. <i>In Vitro</i> Labeling and Processing	35
3. CNS Receptor Localization: Focus on the Hippocampal Formation	41
4. Future Considerations	47
References	48

Chapter 3

Receptor Measurement

Ernest J. Peck, Jr. and Katrina L. Kelner

1. Introduction	53
2. Criteria for Receptors	53
2.1. Finite Binding Capacity	53
2.2. Appropriate Affinity	54
2.3. Ligand Specificity	54
2.4. Target Specificity	54
2.5. Correlation With Biological Response	55
3. Analysis of Simple and Complex Binding Systems	55
3.1. Single-Component Systems	55
3.2. Complex Binding Systems	56
4. Methods for Receptor Measurement	65
4.1. Steroid Receptors	66
4.2. Neurotransmitter Receptors	69
5. Conclusions	74
References	74

Chapter 4

Rapid Enzyme Inactivation

Robert H. Lenox, G. Jean Kant, and James L. Meyerhoff

1. Introduction	77
2. Freezing Methods	78
2.1. Brain <i>in Situ</i>	78
2.2. Brain <i>ex Situ</i>	81

3. Microwave Methods	84
3.1. Microwave Radiation	84
3.2. Microwave Inactivation Systems	85
3.3. Neurochemical Studies	86
3.4. Limitations	93
3.5. Summary	98
4. Future Techniques	99
References	100

Chapter 5

Radioenzymatic Analyses

Stephen R. Philips

1. Introduction	103
2. Catecholamines, Precursors, and Metabolites	103
2.1. Norepinephrine, Epinephrine, and Dopamine	103
2.2. DOPA	110
2.3. Normetanephrine	110
2.4. Dihydroxymandelic Acid, Dihydroxyphenylacetic Acid, and Dihydroxyphenylglycol	111
3. Serotonin	113
4. Acetylcholine	115
5. Histamine	117
6. Octopamine and Phenylethanolamine	120
7. Trace Amines	122
7.1. β -Phenylethylamine	123
7.2. Tyramine	123
7.3. Tryptamine	124
8. Taurine	125
9. Glutamine	125
10. Advantages and Limitations	126
11. Conclusions	127
References	127

Chapter 6

Two-Dimensional Polyacrylamide Gel Electrophoresis of Proteins

David L. Wilson

1. Introduction	133
2. Before and After 2D-PAGE: Limitations	133
3. Details of the 2D-PAGE Technique	136
3.1. Sample Preparations	136
3.2. The First Dimension: pI	137
3.3. The Second Dimension: Molecular Weight	137
3.4. The Third Dimension: Abundance	138

3.5. Comigration Analysis	141
3.6. Continued Analysis of Proteins following 2D-PAGE	142
4. Troubleshooting 2D-PAGE	142
4.1. No Spots on Gel after Staining	142
4.2. Staining Pattern Has Streaks in Isoelectric Focusing Direction (Horizontal Streaks)	143
4.3. Staining Pattern Has Streaks in Molecular Weight Direction (Vertical Streaks)	143
4.4. Proteins at Basic End of Gel Show Some Streaking and Variability from Gel to Gel	143
4.5. Isoelectric Focusing Pattern (pH Gradient) Is Shifted or Distorted	143
4.6. Molecular Weight Distribution Has Shifted	144
4.7. X-Ray Film Has Spurious Marks, Lines, or Background Exposure	144
4.8. Uneven Tracking Dye Front during Second-Dimension Run	144
4.9. Distorted Spots	144
5. Two-Dimensional PAGE as a Tool for Neuroscience	144
6. Conclusions	145
References	145

Chapter 7

The Identification of Subcellular Fractions of the CNS

Suella W. Henn and Fritz A. Henn

1. Introduction	147
2. Synaptosomes	147
2.1. Review of Preparative Methods	147
2.2. Criteria for Purity	149
3. Synaptosomal Plasma Membranes	153
4. Postsynaptic Junctional Densities	154
5. Nuclei	155
6. Mitochondria	156
7. Microsomes	157
8. Lysosomes	157
9. Plasma Membranes	158
10. Conclusion	159
References	159

Chapter 8

Cell Isolation

Jørgen Clausen

1. Introduction	163
2. Cellular Composition of CNS and PNS	164

3. General Principles for Cell Isolation	166
3.1. Advantages and Pitfalls of Using Enriched Brain Cell Fractions	166
3.2. Factors Determining the Possibilities for Separation and Isolation of Brain Cells	167
3.3. Principles for Separation of Dissociated Brain Cells	168
4. Characterization of Separated and Isolated Brain Cells	177
4.1. Morphology	177
4.2. Biochemical Criteria	177
5. Application of Procedures for Isolation of Brain Cells	178
6. Conclusion	178
References	179

Chapter 9

Principles of Compartmentation

N. M. van Gelder

1. Introduction	183
2. Anatomic Compartmentation	184
2.1. Blood-Brain Barrier Systems	184
2.2. Oligodendrocytes	185
2.3. Astrocytes	185
2.4. Neurons	185
3. Biochemical Compartmentation	189
3.1. The Separation of Metabolic Pools	190
3.2. The Separation of Endogenous Pools	193
4. Tissue Compartmentation	196
4.1. Intact Animal	196
4.2. Tissue Preparations	198
References	202

Chapter 10

Diagnosis of Hereditary Neurological Metabolic Diseases

A. Lowenthal, N. Chamoles, K. Adriaenssens, and R. Humbel

1. Introduction	207
2. Methodology	209
2.1. Screening	209
2.2. Identification of the Disease	209
3. Physiopathological Considerations	210
4. Classification of Human Metabolic Diseases in Neurology	211
4.1. The Aminoacidopathies	211
4.2. Carbohydrate Disorders	214
4.3. Diseases of Glycoconjugation (Mucopolysaccharidoses, Mucolipidoses, and Oligosaccharidoses)	215
4.4. Disorders of Lipids and Fatty Acids	217

4.5. Organic Acidurias: Errors of Metabolism That Cause Acute Life-Threatening Illness in the First Weeks of Life	218
4.6. Diseases with Metal or Trace Element Accumulation	220
4.7. Diseases That Are Difficult to Classify	220
5. Therapeutic Conclusions	221
5.1. Diet Deprived of Accumulated Substances	221
5.2. Vitamins	221
5.3. Detoxification Therapies	221
5.4. Treatment of Organic Acidurias	222
5.5. Substitution Therapy by Enzymes	222
5.6. Prevention	222
References	222

Chapter 11

Human Brain Postmortem Studies of Neurotransmitters and Related Markers

E. D. Bird and L. L. Iversen

1. Introduction and Historical Aspects	225
2. Selection, Collection, Handling, and Storage	226
3. Dissection	228
4. Assay Procedures	230
5. Postmortem Stability	231
5.1. Methods for Assessing Postmortem Stability	232
5.2. Results Obtained	233
6. Compilation of Control Data	236
7. Factors Influencing Postmortem Neurochemical Data	237
7.1. Age-Related Trends	238
7.2. Circadian Changes	240
7.3. Agonal State	241
7.4. Postmortem and Storage Interval	243
7.5. Drug Treatment	245
7.6. Tissue Volume Changes	246
7.7. Other Factors	246
8. Disease States	246
8.1. Degenerative Disorders	246
8.2. Nondegenerative Disorders: Schizophrenia	249
References	249

Chapter 12

Neurological Mutants

Nicole Baumann and François Lachappelle

1. Introduction	253
2. Neurological Mutants in Nonrodent Species	254

2.1. Epilepsy in Baboons	254
2.2. Inborn Errors of Metabolism	255
3. Murine Models	257
3.1. Dysraphic Disorders	260
3.2. Hydrocephalus	260
3.3. Cerebellar Malformations	260
3.4. Epilepsies	263
3.5. Myelin Defects	263
3.6. Neuromuscular Disorders	270
3.7. Inherited Metabolic Disorders	272
3.8. Problems Related to the Production and Use of Neurological Mutants	275
References	275

Chapter 13

Analytical Aspects of the Pharmacokinetics of Psychotropic Drugs

Thomas B. Cooper

1. Introduction	281
2. Pharmacokinetic Background	284
3. Analytical Procedure	285
3.1. Glassware for Collection of Samples and Extraction Procedure	285
3.2. Sample Collection	286
3.3. Storage of Samples	286
3.4. Extraction Procedures	288
3.5. Internal Standards	288
4. Thin-Layer Chromatography	289
5. Gas Chromatography of Tricyclic Antidepressants and Antipsychotics	290
5.1. Flame Ionization Detection	290
5.2. Electron-Capture Detection	290
5.3. Gas Chromatography with Nitrogen-Phosphorus Detection	290
5.4. Gas Chromatography/Mass Spectrometry	291
6. High-Performance Liquid Chromatography	292
7. Radioimmunoassay	293
8. Radioreceptor Assays	294
9. Conclusion	295
References	296

Chapter 14

Perfusion of the Isolated Brain

David D. Gilboe

1. Rationale for Use of Isolated Brains	301
2. Review of Procedures Employed to Isolate the Brain	302

2.1. Isolated Heads	302
2.2. Partially Isolated Brains	303
2.3. Completely Isolated Brains	303
3. Criteria of Viability	304
4. Fluids Used to Perfuse the Brain	304
5. Metabolic Studies	308
5.1. Metabolic Uptake and Efflux with Various Preparations ...	308
5.2. Tissue Metabolism in Various Preparations Under Control Conditions	311
5.3. Metabolic Changes in Blood and Tissue during Hypoxia ...	313
5.4. Tissue Metabolites and Electrolytes during and following Hypoxia	313
6. Pharmacological Studies	316
6.1. Influence of Drugs on Cerebral Metabolism	316
6.2. Drug Effects on Cerebrovascular Resistance	319
6.3. Drug Uptake	320
7. Metabolite Transport	321
7.1. Glucose Transport	321
7.2. Amino Acids	325
7.3. Undirectional Flux of Other Compounds	328
References	328

Chapter 15

Principles and Application of PET in Neuroscience

Jonathan D. Brodie, Nora Volkow, and John Rotrosen

1. Introduction	331
2. Methodology	331
2.1. Preparation of the Radionuclide	331
2.2. Detection of the Radioactivity	332
2.3. Reconstruction Process	335
3. Physiological Studies with PET	336
3.1. Glucose Metabolism	337
3.2. Oxygen Metabolism	339
3.3. Brain Hemodynamics	340
3.4. Brain Chemical Composition	341
4. Functional Studies	341
5. Clinical Applications	342
5.1. Epilepsy	342
5.2. Stroke	343
5.3. Dementia	344
5.4. Huntington's Chorea	344
5.5. Cerebral Tumors	344
5.6. Schizophrenia	345
References	345

8703-92

B1

Chapter 16***Selected Micromethods for Use in Neurochemistry****Volker Neuhoff*

1. Introduction	349
2. Protein Determination with Microliter Volumes	350
2.1. Performance of the Protein Determination	351
2.2. Protein Determination with Unknown Volume and Unknown Concentration	355
3. Glycoprotein Determination with FITC-Labeled Lectins at the Nanogram Range	358
3.1. Practical Performance	358
3.2. Simultaneous Determination of Protein and Glycoprotein ..	362
4. Determination of Sugars in the Picomole Range	362
5. One- and Two-Dimensional Electrophoresis in Polyacrylamide Capillary Gels and Microslab Gels	364
5.1. Electrophoresis in Homogeneous Capillary Gels	364
5.2. Microgradient Gels	367
5.3. Isoelectric Focusing in Capillary Gels	368
5.4. One- and Two-Dimensional Microslab Gel Electrophoresis ..	369
6. Determination of Amino Acids and Related Compounds with Dansyl Chloride in the Picomole Range	379
6.1. Practical Procedure	379
6.2. Microchromatography of Dansyl Amino Acids	382
7. Photometry and Fluorometry of Microgels and Microchromatograms	383
8. Micromethods for Analysis at the Cellular Levels	384
9. Auxiliary Equipment for Microanalysis	384
10. Concluding Remarks	385
References	391

Chapter 17***Mass Spectrometric Analysis of Some Neurotransmitters and Their
Precursors and Metabolites****David A. Durden and Alan A. Boulton*

1. Introduction	397
2. Mass Spectrometry	398
2.1. The Process of Mass Spectrometry	398
2.2. Formation of Ions	401
2.3. Mass Spectrometric Methods	402
2.4. Internal Standards for Quantitative Analysis	403
3. Chromatographic-Mass Spectrometric Methods	404
3.1. Thin-Layer Chromatography-Mass Spectrometry	404
3.2. Gas Chromatography-Mass Spectrometry	404

4. Summaries of Methods	405
4.1. Amines	405
4.2. Acids	412
4.3. Alcohols	418
4.4. Amino Acids	420
5. Appendix	423
References	424

Chapter 18

Gas Chromatography

Ronald T. Coutts and Glen B. Baker

1. Introduction	429
2. Basic Principles of Gas Chromatography	429
2.1. Gas Chromatographic Columns	430
2.2. Detectors	431
3. Derivatization for Analysis by Gas Chromatography	432
4. Analysis of Specific Types of Compounds by Gas Chromatography	433
4.1. Endogenous Arylalkylamines	433
4.2. Acidic and Neutral Metabolites of Endogenous Arylalkylamines	435
4.3. Polyamines	436
4.4. Amino Acids	436
4.5. Acetylcholine and Choline	437
4.6. Lipids	437
4.7. Steroids	438
4.8. Prostaglandins	439
4.9. Carbohydrates	439
4.10. Purine and Pyrimidine Bases, Nucleosides, and Nucleotides	440
4.11. Antidepressants	440
4.12. Neuroleptics	440
4.13. Benzodiazepines	441
4.14. CNS Stimulants and Related Compounds	441
4.15. Barbiturates, Hydantoins, and Related Compounds	442
4.16. Alkaloids	443
5. Advantages and Disadvantages of Gas Chromatography	444
References	445

Chapter 19

High-Performance Liquid Chromatography

Stanley Stein

1. Introduction	449
2. Principles and Practices	449
2.1 HPLC Supports	449
2.2. Efficiency and Selectivity	451