Cellular Proteases and Control Mechanisms

Cellular Proteases and Control Mechanisms

Proceedings of a Glaxo-UCLA Colloquium on Cellular Proteases and Control Mechanisms Held at Lake Tahoe, California February 21-26, 1988

Editor

Tony E. Hugli

Scripps Clinic and Research Foundation

La Jolla, California

Alan R. Liss, Inc. New York

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in the United States of America

Library of Congress Cataloging-in-Publication Data

Glaxo-UCLA Colloquium on Cellular Proteases and Control Mechanisms (1988 : Lake Tahoe, Calif)
Cellular proteases and control mechanisms : proceedings of a Glaxo-UCLA Colloquium on Cellular Proteases and Control Mechanisms, held at Lake Tahoe, California, February 21–25, 1988 / editor, Tony E Hugli.
p. cm. — (UCLA symposia on molecular and cellular biology new ser., v. 104)
Includes bibliographies and index.
ISBN 0-8451-4703-X
1. Proteolytic enzymes—Physiological effect—Congresses 2. Proteolytic enzymes—Inhibitors—Congresses I Hugli, T E (Tony E.) II. Title III Series
[DNLM: 1. Peptide Hydrolases—physiology—congresses 2 Protease Inhibitors—pharmacology—congresses W3 U17N new ser v 104 / QU 136 G552c 1988]
QP609.P78G53 1988
574.19'256—dc20
DNLM/DLC
for Library of Congress

89-8202
CIP

Cellular Proteases and Control Mechanisms

Pages 1–140 of this volume are reprinted from the Journal of Cellular Biochemistry, Volumes 38, 39 and 40. The Journal is the only appropriate literature citation for the articles printed on these pages. The page numbers in the table of contents, contributors list, and index of this volume correspond to the page numbers at the foo of these pages.

The table of contents does not necessarily follow the pattern of the plenary sessions. Instead, it reflects the thrust of the meeting as it evolved from the combination of plenary sessions, poster sessions, and workshops, culminating in the final collection of invited papers, submitted papers, and workshop summaries. The order in which articles appear in this volume does not follow the order of citation in the table of contents. Many of the articles in this volume were published in the Journal of Cellular Biochemistry, and they are reprinted here. These articles appear in the order in which they were accepted for publication and then published in the Journal. They are followed by papers which were submitted solely for publication in the proceedings.

Contributors

Foluso Adebodun, Department of Chemistry, Rutgers, The State University of New Jersey, Newark, NJ 07102 **[129]**

B. Ashe, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

R. Beckmann, Laboratory for Clinical-Experimental Physiology, Department of Medical Physiology, University of Vienna, A-1090 Vienna, Austria **[149]**

B.R. Binder, Laboratory for Clinical-Experimental Physiology, Department of Medical Physiology, University of Vienna, A-1090 Vienna, Austria **[149]**

Guy Boileau, Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada **[159]**

R.J. Bonney, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

Eugene Braunwald, Departments of Medicine and Radiology, Brigham and Women's Hospital, Departments of Medicine and Radiology, Beth Israel Hospital, and Harvard Medical School, Boston, MA 02115 **[25]**

G. Christ, Laboratory for Clinical-Experimental Physiology, Department of Medical Physiology, University of Vienna, A-1090 Vienna, Austria **[149]**

Patricia C. Come, Departments of Medicine and Radiology, Beth Israel Hospital, Boston, MA 02215; and Harvard Medical School, Boston, MA 02115 **[25]**

Charles S. Craik, Departments of Pharmaceutical Chemistry, Biochemistry and Biophysics, University of California, San Francisco, CA 94143 **[67]**

Philippe Crine, Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada **[159]**

Dennis D. Cunningham, Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA 92717 **[57]**

P. Davies, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

Douglas L. Dawley, Thoracic Clinic, Portland, OR 97213 **[25]**

P. Dellea, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

J. Deutinger, Second Department of Obstetrics and Gynecology, University of Vienna, A-1090 Vienna, Austria **[149]**

Alain Devault, Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada **[159]**

J. Doherty, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

C. Dorn, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

Ann H. Erickson, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599-7260 **[117]**

Luke B. Evnin, Departments of Biochemistry and Biophysics, University of California, San Francisco, CA 94143 **[67]**

P. Finke, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

D. Fletcher, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

The numbers in brackets are the opening page numbers of the contributors' articles.

D. Frankenfield, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486 [49]

Lloyd D. Fricker, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461 [1]

Uli Fruth, Max-Planck-Institute for Immunobiology, Freiburg, Germany [87]

David Gies, Department of Cell Genetics, Genentech, Inc., South San Francisco, CA 94080 [79]

Samuel Z. Goldhaber, Departments of Medicine and Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 [25]

Cornelia M. Gorman, Department of Cell Genetics, Genentech, Inc., South San Francisco, CA 94080 [79]

David Gurwitz, Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA 92717 [57]

W. Hagmann, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 [49]

K. Hand, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 [49]

Masakazu Hatanaka, Institute for Virus Research, Kyoto University, Sayko, Kyoto 606, Japan [101]

John Heit, Mayo Clinic, Rochester, MN 55905 [25]

Maria A. Hernandez, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 [35]

Jeffrey N. Higaki, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 [67]

Tony E. Hugli, Scripps Clinic and Research Foundation, La Jolla, CA 92037 [xv]

Michael A. Innis, Cetus Corporation, Emeryville, CA 94608 [141]

Elizabeth W. Jones, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213 [141]

Frank Jordan, Department of Chemistry, Rutgers, The State University of New Jersey, Newark, NJ 07102 [129]

Helen Kado-Fong, Department of Pharmacological Sciences, Genentech, Inc., South San Francisco, CA 94080 [79]

Chih-Min Kam, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 [35]

Nobuhiko Katunuma, Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Tokushima 770, Japan [13]

Craig M. Kessler, Department of Medicine, George Washington University Medical Center, Washington, DC 20037 [25]

Hiroshi Kido, Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Tokushima 770, Japan [13]

Ducksoo Kim, Departments of Medicine and Radiology, Beth Israel Hospital, Boston, MA 02215; and Harvard Medical School, Boston, MA 02115 [25]

J.C. Kirchheimer, Laboratory for Clinical-Experimental Physiology, Department of Medical Physiology, University of Vienna, A-1090 Vienna, Austria [149]

Michael D. Kramer, Universitätshautklinik, Heidelberg, Germany [87]

Joseph Loscalzo, Departments of Medicine and Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 [25]

Bernard Malfroy, Department of Pharmacological Sciences, Genentech, Inc., South San Francisco, CA 94080 [79]

John E. Markis, Departments of Medicine and Radiology, Beth Israel Hospital, Boston, MA 02215; and Harvard Medical School, Boston, MA 02115 [25]

A. Maycock, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 [49]

Michael F. Meyerovitz, Departments of Medicine and Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 **[25]**

Charles M. Moehle, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213; present address: National Institutes of Health, Bethesda, MD 20892 **[141]**

R. Mumford, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

Seok Hyun Nam, Institute for Virus Research, Kyoto University, Sayko, Kyoto 606, Japan **[101]**

Lakshmi Narasimhan, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 **[35]**

Janelle A. Noble, Cetus Corporation, Emeryville, CA 94608; present address: Department of Biochemistry, University of California-Berkeley, Berkeley, CA 94720 **[141]**

T. Nolan, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486 **[49]**

Joszef Oleksyszyn, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 **[35]**

D. Osinga, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

J. Anthony Parker, Departments of Medicine and Radiology, Beth Israel Hospital, Boston, MA 02215; and Harvard Medical School, Boston, MA 02115 **[25]**

James C. Powers, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 **[35]**

Marlot Prester, Max-Planck-Institute for Immunobiology, Freiburg, Germany **[87]**

A. Reinthaller, Second Department of Obstetrics and Gynecology, University of Vienna, A-1090 Vienna, Austria **[149]**

L. Schaeffer, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486 **[49]**

Peter R. Schofield, Department of Developmental Biology, Genentech, Inc., South San Francisco, CA 94080 **[79]**

Andrew P. Selwyn, Departments of Medicine and Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 **[25]**

S. Shah, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 **[49]**

G.V.R.K. Sharma, Department of Medicine, Veterans Administration Medical Center at Brockton/West Roxbury, West Roxbury, MA 02132; and Harvard Medical School, Boston, MA 02115 **[25]**

Markus M. Simon, Max-Planck-Institute for Immunobiology, Freiburg, Germany **[87]**

G. Tatra, Second Department of Obstetrics and Gynecology, University of Vienna, A-1090 Vienna, Austria **[149]**

Toshihisa Ueda, School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 **[35]**

John R. Vasquez, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 **[67]**

Douglas E. Vaughan, Departments of Medicine and Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 **[25]**

Carol A. Woolford, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213 **[141]**

Preface

Advances in basic research areas such as the proteases have been revolutionized in recent years by molecular biologic techniques. This new technology has permitted molecular characterization of many cell products not readily available using classical protein methodologies. Trace proteases from cellular sources have been studied with new intensity in recent years. Therefore, data that provide an adequate understanding of protease processing, activation, and regulation events are just now becoming available.

Applications of this new information in the clinical arena are already being undertaken as therapeutic protocols for enzyme/inhibitor replacement are explored. Also, it is hoped that recombinant inhibitor molecules will be effectively used in treating a spectrum of diseases from progressive chronic conditions such as emphysema to acute forms such as myocardial infarction.

It was this broad-based front of research activity in the field of proteases that led to the organization of a conference held in Lake Tahoe, California, February 21–26, 1988. The meeting **Cellular Proteases and Control Mechanisms** was designed to bring together a representative cross section of scientists working in the field of proteases and protease inhibitors. The goal was to examine proteases in molecular architectural, molecular biologic, regulatory, and clinical terms. This proceedings volume contains a collection of conference presentations that exemplify these various areas. A feature of this conference that may be uncharacteristic of other UCLA colloquia was its bringing the latest clinical data concerning effective therapeutic uses of protease inhibitors and their complications to the attention of leading basic researchers in this field.

Effective utilization of proteases or protease inhibitors in commercial or clinical applications will depend on the quantity of basic knowledge available. It is important not only to disseminate current information but also to examine whether the direction of effort will assist in or maximize applications for the treatment and prevention of disease. It was this goal that guided the selection of scientists, who presented their results in an atmosphere of active discussion. It was the intent of the organizers to encourage clinical researchers to participate by identifying problems that could perhaps be solved in the basic research laboratory and to focus on the current limitations of using proteases or inhibitors as therapeutic agents. In this regard, the meeting proved to be a lively and constructive interchange of ideas.

The volume consists of 15 articles representing four topic areas from the colloquium. Two general sections deal with various characterizations of cellular proteases and inhibitors. These sections were designed to address the diversity of cellular proteases and protease inhibitors and to identify the tremendous technical difficulty

that one faces when studying trace materials such as cellular products. The next section deals with the latest technical approaches using molecular biology to obtain fundamental information about molecules that are not otherwise easily isolated in quantity. These reports outline molecular details of proteases and regulators of proteases that were not generally obtainable just five years ago. Most of these protein molecules certainly could not be fully characterized without the tools of molecular biology. These articles largely demonstrate the enormous latitude for obtaining new and useful information without the need for classical purification of proteins from a cellular source. Finally, in Section IV we have an article that serves to define the potential of protease regulators in therapeutic treatment of disease. It is this type of approach that validates the need to continue the basic exploration of protease structure, function, and design.

We wish to express our gratitude to Robin Yeaton and Jacqueline Wester, who made major contributions to the success of the colloquium by offering continued assistance both before and during the meetings in Tahoe. We are also indebted to Alice B. Clagett for her role in supervising the manuscript review process.

We gratefully acknowledge the support of Glaxo, Inc., and The Directors' Fund, sponsors of the colloquium, and special thanks go to Applied Biosystems, Inc., for providing funds to support the Keynote Address, and to Vestar, Inc.

Tony E. Hugli

Contents

III. MOLECULAR BIOLOGY OF CELLULAR PROTEASES AND THEIR REGULATORS

IV. THERAPEUTIC APPLICATIONS OF PROTEASE INHIBITORS

Journal of Cellular Biochemistry 38:279–289 (1988)
Cellular Proteases and Control Mechanisms 1–11

Activation and Membrane Binding of Carboxypeptidase E

Lloyd D. Fricker

Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461

Carboxypeptidase E (CPE)is a carboxypeptidase B-like enzyme that is thought to be involved in the processing of peptide hormones and neurotransmitters. Soluble and membrane-associated forms of CPE have been observed in purified secretory granules from various hormone-producing tissues. In this report, the influence of membrane association on CPE activity has been examined. A substantial amount of the membrane-associated CPE activity is solubilized upon extraction of bovine pituitary membranes with either 100 mM sodium acetate buffer (pH 5.6) containing 0.5% Triton X-100 and 1 M NaCl, or by extraction with high pH buffers (pH > 8). These treatments also lead to a two- to threefold increase in CPE activity. CPE extracted from membranes with either NaCl/Triton X-100 or high pH buffers hydrolyzes the dansyl-Phe-Ala-Arg substrate with a lower K_m than the membrane-associated CPE. The V_{max} of CPE present in extracts and membrane fractions after the NaCl/Triton X-100 treatment is twofold higher than in untreated membranes. Treatment of membranes with high pH buffers does not affect the V_{max} of CPE in the soluble and particulate fractions. Pretreatment of membranes with bromoacetyl-D-arginine, an active site-directed irreversible inhibitor of CPE, blocks the activation by NaCl/Triton X-100 treatment. Thus the increase in CPE activity upon extraction from membranes is probably not because of the conversion of an inactive form to an active one, but is the result of changes in the conformation of the enzyme that effect the catalytic activity.

Key words: enkephalin convertase, carboxypeptidase H, carboxypeptidase B-like, neuropeptide biosynthesis

Many peptide hormones and neurotransmitters are initially produced as large precursors that must be enzymatically processed into the bioactive peptides [1]. The processing sites are usually pairs of basic amino acids, and the sequential action of a trypsin-like endopeptidase and a carboxypeptidase B-like exopeptidase produces the bioactive peptides. Carboxypeptidase E (CPE, EC 3.4.17.10, also designated enkephalin convertase and carboxypeptidase H) is a carboxypeptidase B-like enzyme associated with the biosynthesis of numerous peptide hormones and neurotransmitters. CPE is present in many tissues where peptide biosynthesis occurs, such as brain, pituitary,

Received May 3, 1988; accepted June 9, 1988.

and adrenal medulla [2–8]. Within the pituitary, adrenal medulla, and an insulin-producing tumor, CPE is associated with the peptide hormone-containing secretory granule fraction [3,6–8], which is the putative site of peptide processing. CPE is maximally active at pH 5.6 [3,6], the intragranular pH of pituitary and adrenal medulla secretory granules [9,10].

Within the secretory granules, CPE is present in several forms. One form is soluble upon extraction of lysed granules with low ionic strength buffers, such as 10 mM sodium acetate, pH 6 [3]. A small amount of the membrane-associated CPE activity is solubilized by 1 M NaCl [11]. Some of the remaining membrane-associated CPE activity is solubilized with buffers containing both 1 M NaCl and 0.5% Triton X-100 in 10 mM sodium acetate, pH 6 [12]. The different forms of CPE have been purified to apparent homogeneity using affinity and ion exchange chromatography [12,13]. Both the soluble and membrane associated forms of CPE have similar enzymatic and physical properties [3,12,13]. The only detectable difference between the purified forms of CPE is the apparent molecular weight on SDS polyacrylamide gels, with the soluble form (50,000 daltons) slightly smaller than the form extracted from membranes by NaCl/Triton X-100 (52,000 daltons). Both forms have the same amino acid sequence of the N-terminal region [14].

Recently a cDNA clone encoding bovine CPE has been isolated and sequenced [14]. Southern blot analysis of bovine genomic DNA indicates that a single gene encodes CPE. Northern blot analysis of bovine pituitary mRNA shows several species that hybridize with the cDNA probes [14]. One of these species accounts for $> 95\%$ of the CPE mRNA, and the other RNA species presumably arises from alternate polyadenylation sites within the 3′ untranslated region [14]. These results suggest that the differences between the soluble and membrane forms of CPE are the result of post-translational modifications of a single precursor protein. This protein does not contain hydrophobic regions that would be predicted to form transmembrane-spanning domains. Instead, a potential amphipathic helix located within the C-terminal region may assist in the association with membranes [14,15], although there is no direct evidence for this possibility.

Antisera raised against the purified soluble form of CPE binds to both soluble and membrane-extracted forms with equal affinity [16]. When examined by either radioimmunoassay or immunoblot techniques, comparable amounts of CPE immunoreactivity are present in soluble and membrane fractions of adrenal medulla chromaffin granule lysates [17,18]. However, when similar fractions are examined for carboxypeptidase activity, there is significantly more CPE activity in the soluble extracts [3,17]. This finding suggests that the specific activity of the soluble form is greater than the specific activity of the membrane-bound form. Since CPE purified to apparent homogeneity from either soluble or membrane extracts has a similar specific activity [12], it is likely that the membrane form is less catalytically active when bound to membranes. In the present study, this possibility has been tested by measuring the enzymatic activity and kinetic parameters of the membrane form of CPE both before and after solubilization, using different extraction conditions.

MATERIALS AND METHODS

Frozen bovine pituitaries were purchased from Pelfreeze. Membranes were prepared by homogenizing (Brinkman Polytron) the pituitaries in 5 volumes 100 mM

NaAc, pH 5.6. The homogenate was centrifuged for 1 hour at 150,000*g*. The supernatant was removed, and the pellet was resuspended in 100 mM NaAc, pH 5.6, and centrifuged at 150,000*g* for 1 hour. After two extractions with 100 mM NaAc, the pellet was similarly extracted twice with 1 M NaCl in 100 mM NaAc buffer, pH 5.6. The pellet was resuspended in H_2O, frozen (−20°C), thawed, and centrifuged as above. The supernatant was removed, and the pellet was resuspended in 10 mM NaAc buffer, pH 5.6. This pellet is referred to as the "membrane" fraction.

Carboxypeptidase E activity was assayed using dansyl-Phe-Ala-Arg, as previously described [13]. In a typical assay, tissue extract, NaAc buffer, pH 5.6 (50–100 mM final concentration), and substrate (100 μM final concentration) were combined in a total volume of 250 μl. The samples were incubated at 37°C for 20 min, and the reaction was stopped with 100 μl 0.5 M HCl. Chloroform (2 ml) was added, the tubes were mixed and centrifuged (1 minute at 1,000 rpm in a Beckman TJ-6), and the fluorescence in the lower organic phase was determined in a Perkin-Elmer LS-3 fluorimeter. Substrate is insoluble in chloroform, whereas the product (dansyl-Phe-Ala) is highly soluble in this solvent [13]. Standard curves using dansyl-Phe-Ala were used to convert the fluorimeter readings into nmoles product. All determinations were performed in triplicate. Carboxypeptidase E activity was calculated from the difference between enzymatic activity measured in the presence and absence of 1 μM GEMSA. These conditions have been previously shown to be specific for CPE [12,19,20]. In some cases, the CPE activity has been adjusted for the number of pituitary glands used in the membrane preparation, which allows the results of separate experiments to be compared.

RESULTS

As previously reported [3,11,12], bovine pituitary glands contain several forms of CPE activity. One of these activities is observed in the supernatant of pituitaries extracted with 10 mM NaAc, pH 5.6 (Fig. 1). Repeated homogenization and extraction with either 10 or 100 mM NaAc, pH 5.6, does not extract the majority of the membrane-associated enzymatic activity. High salt buffers (1 M NaCl/100 mM NaAc, pH 5.6) extract some of the membrane-associated activity. The addition of 0.5% Triton X-100 to the high salt buffer solubilizes a substantial amount of the membrane-bound activity. However, some CPE activity remains associated with the membranes even after a second extraction with 0.5% Triton X-100 in high salt buffer (Fig. 1).

To investigate whether solubilization of the membrane-associated form leads to an increase in the enzymatic activity, pituitary membranes were prepared by repeated homogenization and extraction of bovine pituitaries with 100 mM NaAc, pH 5.6, and then 1 M NaCl in the same buffer. Treatment of these washed membranes with 0.5% Triton X-100 in the high salt buffer, followed by centrifugation, solubilizes a substantial amount of the CPE activity (Fig. 2). Quantitation of this enzymatic activity shows there is more activity in the supernatant than is present in the membranes before treatment with NaCl/Triton X-100. The level of CPE activity in the membranes after extraction with NaCl/Triton X-100 is only slightly lower than the level of activity present in the homogenate before the extraction. The net result is a two- to threefold increase in the total CPE activity upon NaCl/Triton X-100 treatment of membranes, with 65% of the CPE activity solubilized by the treatment. Centrifugation of untreated

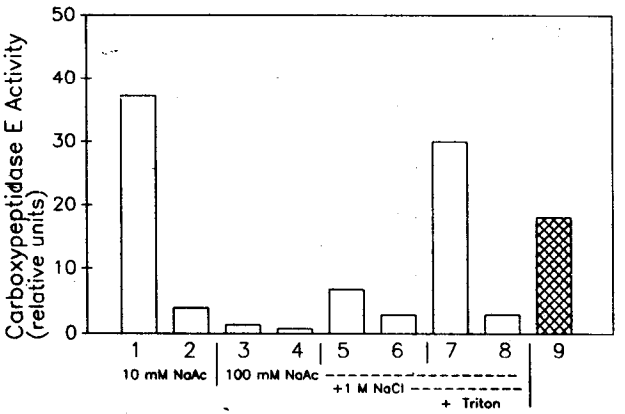

Fig. 1. Extraction of CPE activity from bovine pituitary. One whole pituitary was homogenized (Polytron) in 10 ml of 10 mM NaAc buffer, pH 5.6, and then centrifuged at 50,000*g* for 10 min. The supernatant was removed (**column 1**) and the pellet resuspended in the same buffer. The homogenate was centrifuged as above and the supernatant removed (**column 2**). The pellet was extracted in a similar manner with 100 mM NaAc, pH 5.6 (**columns 3, 4**), with 1 M NaCl in 100 mM NaAc, pH 5.6 (**columns 5, 6**), and then with 0.5% Triton X-100 in the 1 M NaCl/100 mM NaAc buffer, pH 5.6 (**columns 7, 8**). The pellet was resuspended in 10 ml 100 mM NaAc, pH 5.6 (**column 9**), and aliquots of each fraction were assayed for CPE activity as described in Materials and Methods.

membrane homogenates does not produce an increase in CPE activity, and all of the enzymatic activity is recovered in the membrane fraction.

Treatment of bovine pituitary membranes with high pH buffers (100 mM $NaHCO_3$, pH 9) also leads to a solubilization and elevation of the CPE activity (Fig. 2). As with the NaCl/Triton X-100 extraction, the level of CPE activity in the supernatant following the high pH treatment is greater than the level of activity in the untreated membranes. The CPE activity remaining in the membranes is lower after extraction with $NaHCO_3$ than with NaCl/Triton X-100. The total CPE activity is approximately twofold higher after $NaHCO_3$ extraction, compared with the level in the membranes before extraction.

Treatment of pituitary membranes with buffers other than $NaHCO_3$ also leads to a solubilization of the CPE activity (Fig. 3). Sodium borate buffers, ranging from pH 8 to pH 10, are comparable to $NaHCO_3$ in their effect on the membrane association and activity of CPE. The increase in enzymatic activity in the soluble extracts is accompanied by a decrease in enzymatic activity remaining in the membranes. However, the sum of the extracted and membrane-bound activities is two- to threefold higher than when membranes are treated with NaAc, pH 5.6 (Fig. 3). Sodium acetate buffer at pH 6.0 is comparable to the same buffer at pH 5.6, with all of the enzymatic activity remaining membrane-bound. Interestingly, treatment of membranes with sodium phosphate buffers of pH 5.6 or 6.0 leads to a small increase in the activity present in the soluble extracts. Sodium phosphate buffers in the pH 6–8 range are more effective in solubilizing the membrane-bound activity than the lower pH buffers, but are not as effective as the sodium borate buffers. Tris-chloride buffers of pH 7.4–8.4 vary in effectiveness, with the lower pH buffer comparable to sodium phosphate and the higher pH buffers comparable to sodium borate and sodium