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STIFTUNG PROFESSOR DR. MAX CLOËTTA

Heft Nr. 11

«Peptides and proteins as messengers
in the communication between cells and organs»

«Regulation of insulin release by intracellular calcium»

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PROFESSOR DR. MAX CLOËTTA

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INHALT

Vorwort des Präsidenten des Stiftungsrates, Herrn Dr. Hans W. Kopp 5

Votum von Herrn alt Bundesrat Dr. Hans Hürlimann zum Thema: «Stiftung und Staat» 9

Laudatio von Herrn Professor Jeremias Kägi auf den ersten Preisträger, Herrn Professor Peter Böhlen 13

Urkunde für Herrn Professor Peter Böhlen 15

Kurzbiographie von Herrn Professor Peter Böhlen 17

Festvortrag von Herrn Professor Peter Böhlen mit dem Thema: «Peptides and proteins as messengers in the communication between cells and organs» 21

Laudatio von Herrn Professor Alex F. Muller auf den zweiten Preisträger, Herrn PD Dr. Claes B. Wollheim 52

Urkunde für Herrn PD Dr. Claes B. Wollheim 55

Kurzbiographie von Herrn PD Dr. Claes B. Wollheim 57

Festvortrag von Herrn PD Dr. Claes B. Wollheim mit dem Thema: «Regulation of insulin release by intracellular calcium» 59

Übersicht über die bisher erschienenen Publikationen der Schriftenreihe Stiftung Professor Dr. Max Cloëtta 85

Ehrentafel der Preisträger 87

VORWORT

Oft ist vom «Werkplatz Schweiz», auch vom «Finanzplatz Schweiz» oder vom «Denkplatz Schweiz» die Rede. Kürzlich aber fand sich in der «Neuen Zürcher Zeitung» (in Nr. 220 vom 21. September 1983, auf Seite 35) ein Alarmruf unter dem Titel «Abbau des Forschungsplatzes Schweiz?». Der Beitrag stammte aus der Feder von Herrn Professor Dr. Alfred Pletscher, Präsident des Forschungsrats des Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung und Mitglied des Stiftungsrats der Stiftung Professor Dr. Max Cloëtta. Mit eindrücklichen Formulierungen machte Herr Pletscher darauf aufmerksam, wie sehr die öffentlichen Mittel für die Hochschulforschung beschnitten worden sind und weiterhin beschnitten zu werden drohen. Der Realwert der Bundesmittel, die dem Nationalfonds zur Verfügung standen, lag 1982 um dreizehn Prozent tiefer als 1980. Rund zweihundert Mitarbeiterstellen, von total etwa 2200, mussten abgebaut werden. Die schon vorgenommenen Kürzungen drohen unbefristet verlängert zu werden, zusätzliche effektive Kürzungen liegen in der Luft.

Die Stiftung Professor Dr. Max Cloëtta hat diese Entwicklungen mindestens in dem Sinn zu spüren bekommen, dass die Zahl der Gesuche, die uns vorgelegt werden, kontinuierlich ansteigt. Es erscheint dabei oft geradezu unverantwortlich, was für Folgen allein schon in denjenigen Bereichen, in denen die Stiftung tätig ist, ausgelöst oder zunächst riskiert werden. Es kann nur kurzsichtig und gefährlich sein, auf den Gebieten der Grundlagen- und der anwendungsorientierten Forschung, für welche die Mittel des Bundes und der Kantone überwiegend bestimmt sind, die Möglichkeiten zu beschneiden statt auszubauen. Ganz besonders gilt das für ein von seinen natürlichen Ressourcen her an sich «armes» Land wie die Schweiz; und ganz besonders gilt es auch für eine Zeit, die durch tiefgreifende und sehr oft auch überaus rasche Veränderungen gekennzeichnet ist und in der mit Sicherheit den Zug verpasst hat, wer auf irgendeiner Nebenstation saft- und kraftlos und verspätet auch noch aufspringen will.

Die öffentlichen Gemeinwesen disponieren heute mit hohen Steuern

und Abgaben oder indirekt beispielsweise durch die Gesetzgebung über die «zweite Säule» über gewaltige Mittel, die sie selber wieder ausgeben können oder über die sie privaten Personen und Organisationen zumindest die Verfügungsfreiheit ganz oder teilweise nehmen. Diese Mittel fehlen auf der privaten Seite, ohne eine verständnisvolle und nicht allzu kleinliche und bürokratische Förderung vom Staat her können die Hochschulen und ihre Institute nicht auskommen. Eine Stiftung wie die Stiftung Professor Dr. Max Cloëtta kann Lücken füllen, Ergänzungen liefern, da oder dort für eine kleine «Feuerwehrübung» bereit stehen. Oft betrübt und schmerzt es uns, nicht mehr tun zu können; aber die Mittel der Stiftung sind endlich, die Bedürfnisse scheinen es nicht zu sein.

Allzu gering sind immerhin die Mittel der Stiftung auch nicht mehr. Sie wird in nächster Zukunft mit allen ihr vom Stifter, Herrn Dr. Antoine Cloëtta, zur Verfügung gestellten Werten arbeiten können und dann unter normalen Umständen auf jährliche Einkünfte von erheblich über einer Million Franken zählen können. Ein beträchtlicher Teil der Einkünfte soll jeweils zum Vermögen der Stiftung geschlagen werden, erstens weil der Stifter das gewünscht hat und zweitens weil die Geldentwertung durch eine vernünftige Mehrung des Vermögens mindestens ausgeglichen werden soll. Inzwischen sind unter dem Titel der Cloëtta-Preise bereits rund dreihunderttausend Franken zugesprochen worden, läuft das von vielen Seiten warm begrüsste Programm der Schaffung und Finanzierung sogenannter «Cloëtta-Forschungsstellen» und konnten zusätzliche Unterstützungsmassnahmen in Betracht gezogen werden. Jede Möglichkeit des Ausbaus und der Erweiterung freut uns; ich darf an dieser Stelle allen Mitgliedern des Stiftungsrats – in alphabetischer Reihenfolge den Herren Jean-Antoine Cramer, Dr. Alfred Hartmann, Prof. Dr. Alexis Labhart, Prof. Dr. Alfred Pletscher und Prof. Dr. Albert Renold – aufs herzlichste danken für ihre unermüdliche, treue und selbstlose Arbeit im Interesse der Stiftung und damit der Begünstigten der Stiftung.

Kaum zu fassen ist, wie rasch auch für das Leben einer Stiftung die Zeit vergeht. 1983 ist das Jahr der zehnten Preisverleihung. Wir haben Herrn alt Bundesrat Dr. Hans Hürlimann, der dem Stiftungswesen in der Schweiz während seiner Zugehörigkeit zur obersten Exekutive der Schweizerischen Eidgenossenschaft ein verständnis-

voller und wohlgesinnter Schirmherr und ganz besonders für uns ein liebenswürdiger und loyaler Gesprächspartner aus liberaler Aufgeschlossenheit war, gebeten, anlässlich des bescheidenen «Jubiläums» einige persönliche Gedanken zum Thema «Stiftung und Staat» zu formulieren.

Die Preisträger 1983 sind Herr Professor Peter Böhlen (Laudator: Professor Jeremias Kägi) und Herr Dr. Claes B. Wollheim (Laudator: Professor Alex F. Müller). Beiden Gelehrten gilt der Gruss und Glückwunsch der Stiftung; sie, wie alle bisherigen Preisträger, werden die guten Wünsche der Stiftung Professor Dr. Max Cloëtta auch in der Zukunft immer begleiten.

Hans W. Kopp

STIFTUNG UND STAAT

Dr. Hans Hürlimann, alt Bundesrat

Der sehr verdiente Präsident der Stiftung «Professor Dr. Max Cloëtta», Herr Dr. Hans W. Kopp, hat in der Begrüssungsansprache zur achten Preisverleihung im Jahre 1981 zutreffend erwähnt, dass die Stiftung «am Rand mit dem Staat zu tun hat». Diese Feststellung – um nicht zu sagen «Rand»bemerkung – nehme ich zum Anlass, um in der festlichen Stunde der Preisverleihung unter dem Stichwort «Stiftung und Staat» einen rechtlichen und staatspolitischen Bezug aufzuzeigen. Zu diesem Thema skizziere ich drei Gedanken.

1. Der Staat als Hüter

Die Schweiz ist das Land der Stiftungen. Am 31. Dezember 1982 waren im Handelsregister über 23 000 Stiftungen eingetragen: Rund 18 000 dienen der beruflichen Vorsorge zur Deckung des Risikos von Alter, Invalidität und Tod, während die übrigen 5000 Forschung, Kultur und Wohlfahrt fördern. In dieser Zahl sind die Stiftungen des Familien- und Kirchenrechts nicht enthalten.

Zwei Hauptgründe haben zu diesem Stiftungsreichtum beigetragen. Einmal beschränkt sich unser Zivilgesetzbuch auf eine knapp gehaltene Gesetzgebung, welche die Privatautonomie grosszügig respektiert. Solange der Zweck nicht widerrechtlich oder unsittlich ist oder ein Unternehmen vorliegt, für welches das Obligationenrecht die Rechtsform einer Kapitalgesellschaft oder Genossenschaft vorsieht, räumt der Gesetzgeber dem Stifter eine weitgespannte Freiheit ein. Dazu kommt als weiterer Grund die zweckmässige Aufsicht durch den Bund, die Kantone oder die Gemeinden. Diese ist jedoch beschränkt auf die Überwachung einer sorgfältigen Geschäftsführung und die Einhaltung der Vorschriften von Stiftungsurkunde und Stiftungsreglement. Wie das Bundesgericht mehrfach entschieden hat, ist die Stiftungsaufsicht nicht einer Vormundschaftsbehörde gleichgestellt. Sie greift nicht ins Ermessen der Stiftungsorgane ein. Streitigkeiten aufgrund des Stiftungsrechtes werden, sofern nicht eindeutige

Tatbestände für eine Verwaltungsbeschwerde vorliegen, vor den Zivilrichter getragen.
Ein Blick in die Praxis der Aufsichtsbehörden zeigt, dass Zwangsmassnahmen gegenüber Stiftungen die Ausnahme darstellen. Bei Missbräuchen – beispielsweise im Falle offensichtlich zweckwidriger Verwendung der Stiftungsmittel – muss die Aufsichtsbehörde im Interesse des Stifters, aber auch zur Wahrung des Ansehens, das unser Stiftungsrecht geniesst, einschreiten. Unser Zivilgesetzbuch gestattet ferner auf einfache Art die Anpassung des Stiftungszweckes oder der Organisation bei veränderten Verhältnissen, sofern dies dem seinerzeitigen Willen des Stifters entspricht. Der Staat respektiert die Privatautonomie der Stiftung zudem durch Diskretion, obwohl die Gründung einer Stiftung im Handelsamtsblatt publiziert werden muss. Im übrigen aber untersteht auch die Stiftungsaufsicht sowie der Inhalt der Stiftung dem Amtsgeheimnis. So hat beispielsweise das Eidgenössische Departement des Innern, dem rund 800 gemeinnützige Stiftungen zur Aufsicht anvertraut sind, in ein allgemein zugängliches Stiftungsverzeichnis nur jene Stiftungen aufgenommen, deren Stiftungsräte der Veröffentlichung ausdrücklich zustimmten.
Der Staat wird daher zum Helfer der Stiftungsorgane, zum Sachwalter des Stifters, zum Gralshüter des Stifterwillens.

II. Die Stiftung im Dienst des Staates

In unserem Föderativstaat sind Bund, Kantonen und Gemeinden eine Vielfalt von Aufgaben überwiesen. Weder ist das Gemeinwesen in der Lage, noch ist es immer wünschbar, dass alle Aufgaben von der öffentlichen Hand erfüllt werden. Trotzdem bestehen legitime Bedürfnisse der finanziellen Unterstützung und Hilfe. Für die berufliche Vorsorge, die ab Neujahr 1985 aufgrund von Verfassung und Gesetz für die Arbeitnehmer obligatorisch sicherzustellen ist, hat sich das Instrument der Stiftung bereits bewährt. Aber auch für viele Bereiche, welche das Gemeinwesen nicht oder nur teilweise wahrnehmen kann, helfen die Stiftungen in erfreulicher Art. Ein sinnvolles Zusammenleben der Menschen in einer Gemeinschaft setzt Kultur,

Bildung, Forschung, Fürsorge, Hilfe an Benachteiligte und Behinderte naturgemäss voraus. Den vielen Stiftungen in unserem Land haben wir gerade diese Impulse und Unterstützungen zu verdanken. Die Preise, welche heute an verdiente Forscher verliehen werden, zeigen, wie durch Mittel einer Stiftung die medizinische Forschung gefördert und damit kranken und leidenden Menschen geholfen werden kann. Als Vorsteher des Departements des Innern, dem die Stiftungsaufsicht übertragen ist, konnte ich während meiner Amtstätigkeit das segensreiche Wirken von Stiftungen als modernes Mäzenatentum in der Demokratie verfolgen. Der Staat hat daher nicht nur eine Hüterrolle zu erfüllen. Er hat allen Grund, die Leistungen von Stiftern dankbar anzunehmen und Voraussetzungen für das Stiftungswesen zu begünstigen. Darum darf zu Recht der Wunsch ausgesprochen werden: Vivant sequentes!

III. Der Staat als Stifter

Auch der Staat selber hat zum Mittel der Stiftung gegriffen. Um in unserem Bund in besonders empfindlichen Bereichen die staatliche Unterstützung und Förderung ohne politische Einflüsse sicherzustellen, leistet die Eidgenossenschaft an den Schweizerischen Nationalfonds, eine privatrechtliche Stiftung, beträchtliche Mittel zur Unterstützung der Forschung. Wohl werden damit Auflagen verbunden, aber die Entscheidungen über die Finanzierung von Forschungsprojekten – die nationalen Forschungsprogramme ausgenommen – werden von Fachleuten staatsungebunden getroffen.
Auch die Gründung der öffentlich-rechtlichen Stiftung «Pro Helvetia» zur Unterstützung von kulturellen Leistungen im In- und Ausland wurde als zweckmässiges Instrument angesehen, um die Einzelentscheide zur Förderung einer föderativen Kulturpolitik ohne staatlichen Einfluss einem kompetenten Gremium anzuvertrauen. Dieses Konzept wurde vor dem Zweiten Weltkrieg, als uns eine von Diktaturen gesteuerte Kulturpolitik bedrohte, realisiert und durch die eidgenössischen Räte und das Volk in den Jahren 1965 und 1980 ausdrücklich bestätigt.
In neuester Zeit trat der Bund auch als Mitbegründer von Stiftungen

– zum Beispiel für die Stiftung Mikrotechnik in Neuenburg – zusammen mit interessierten Kantonen und Organen der Wirtschaft auf. Der Bund hat damit dem Instrument der Stiftung durch sein eigenes Handeln einen erhöhten Stellenwert zugewiesen.

Mit diesen Gedanken zum Thema «Stiftung und Staat» gratuliere ich dem Stiftungsrat und allen Preisträgern zur zehnten Preisverleihung. Im Andenken an Professor Dr. Max Cloëtta und dessen Sohn, den Stifter Antoine Cloëtta, spreche ich Ihnen, Herr Präsident, und allen Mitgliedern des Stiftungsrates meine Anerkennung aus für das bisherige und zukünftige Wirken im Dienste der medizinischen Forschung.

LAUDATIO
AUF PROFESSOR DR. PETER BÖHLEN

Professor Dr. J. H. R. Kägi

Den Zuwachs an wissenschaftlichen Erkenntnissen verdanken wir heute zu einem grossen Teil der Entwicklung von immer besseren und leistungsfähigeren technischen Hilfsmitteln und Methoden. Dies gilt nicht nur für die Teilchenphysik und Radioastronomie, Wissenschaften, in denen eine weitere Vermehrung unseres Wissens weitgehend vom Bau immer grösserer Maschinen und Teleskope abhängt, sondern auch für die Chemie und die biomedizinischen Wissenschaften, wo die ständige Verfeinerung der experimentellen Verfahren einen immer tieferen Einblick in die Organisation der unbelebten und belebten Natur ermöglicht. Ein schönes Beispiel für diese Entwicklung ist der aufstrebende Wissenszweig der Neuroendokrinologie, wo dank des enorm gesteigerten Auflösungsvermögens der modernen chemischen Analytik in den letzten Jahren eine ganze Anzahl neuer Neurohormone entdeckt und charakterisiert werden konnte. Mit der diesjährigen Preisvergabe an Professor Peter Böhlen, San Diego, ehrt die Cloëtta-Stiftung einen Wissenschafter, der durch bahnbrechende methodische Neuerungen auf dem Gebiet der Peptid- und Proteinanalytik wichtige Voraussetzungen für diese Erfolge geschaffen hat. Der in Bern aufgewachsene und an der Universität Bern als Chemiker ausgebildete Preisträger hat sich schon früh mit der Konzipierung und Ausarbeitung von analytischen und präparativen Mikromethoden für biologisch aktive Aminosäuren und Peptide befasst. Bereits während seiner Postdoktorandenzeit von 1971 bis 1974 bei S. Udenfriend am Roche Institute in Nutley gelang es ihm, durch Markierung mit fluoreszierenden Verbindungen die Nachweisgrenze für Aminosäuren und Peptide gegenüber älteren Methoden um mehrere Grössenordnungen zu senken. In Kombination mit der Hochdruckflüssigphasen-Chromatographie (HPLC) hat diese Neuerung die Peptidanalytik revolutioniert und Möglichkeiten zur Auffindung und Charakterisierung von neuen, in tierischen Geweben nur in geringsten Mengen vorkommenden physiologisch aktiven Peptiden eröffnet.

Am Salk-Institut, wo Herr Böhlen seit 1978 als Mitarbeiter von R. Guillemin (Nobelpreis für Medizin 1977) tätig ist, hat er es verstanden, mit diesem mikrochemischen Werkzeug eine reiche wissenschaftliche Ernte einzubringen. So gelang es ihm, aus dem Zwischenhirn des Schafes ein Peptid mit Somatostatinaktivität (Somatostatin 28) zu isolieren, das eine besonders starke Hemmwirkung auf die Ausschüttung von Wachstumshormon und von Insulin und Glucagon aus den hormonbildenden Zellen hat. Mit gleichen Methoden konnten auch in Keimdrüsen Peptide identifiziert werden, die die Ausschüttung von gonadotropen Hormonen in der Hypophyse regulieren. Ein eigentlicher Test für die Leistungsfähigkeit seiner Methoden war ferner die Auffindung des γ_1-Melanocyten-stimulierenden Hormons in der Rinderhypophyse, eines bislang nicht bekannten Hormons, dessen Existenz aber aus der Struktur des Gens für Proopiomelanocortin vorausgesagt worden war. Das bisher wohl spektakulärste Ergebnis von Böhlens Bemühungen ist jedoch die Reindarstellung und vollständige chemische Charakterisierung eines Polypeptids, dessen biologische Aktivität identisch ist mit jener des lange gesuchten Neurohormons Somatoliberin, das die Freisetzung von Wachstumshormon aus der Hypophyse anregt. Das aus einem seltenen menschlichen Tumor isolierte Peptid enthält 44 Aminosäuren und ist damit das bisher grösste Neurohormon.
Zusammenfassend lässt sich sagen, dass Herr Böhlen durch seine mikropräparativen und mikroanalytischen Methoden das ganze Gebiet der biologisch aktiven Peptide und Hormone erstmals einer systematischen Untersuchung zugänglich gemacht hat. Die grosse Bedeutung dieser Leistung für die Erforschung von weiteren durch Peptide vermittelten interzellulären Regulations- und Kommunikationsprozessen in multizellulären Organismen ist offensichtlich. In Anerkennung seines Beitrags zur Kenntnis von biologisch aktiven Peptiden wird Herrn Böhlen der Cloëtta-Preis 1983 verliehen. Wir gratulieren und wünschen ihm weiterhin Erfolg.

DER CLOËTTA-PREIS 1983
WIRD

HERRN PROFESSOR DR. PHIL. NAT.

PETER BÖHLEN

GEBOREN 1943

DERZEIT ASSOCIATE RESEARCH PROFESSOR
IN NEUROENDOCRINOLOGY
AM SALK INSTITUTE FOR BIOLOGICAL STUDIES,
SAN DIEGO, CALIFORNIA, USA
IN ANERKENNUNG SEINER GRUNDLEGENDEN
BEITRÄGE ZUR ENTWICKLUNG UND
ZUM EINSATZ ULTRAMIKROANALYTISCHER
METHODEN IN DER
NEUROENDOKRINOLOGIE VERLIEHEN.

ZÜRICH, 21. NOVEMBER 1983

FÜR DEN STIFTUNGSRAT:

DER PRÄSIDENT:

DER VIZEPRÄSIDENT:

DAS MITGLIED:

Professor Peter Böhlen

CURRICULUM VITAE
of Professor Dr. Peter Böhlen

Date of Birth:	January 10, 1943
Place of Birth:	Zurich, Switzerland
Sex:	Male
Dependents	Sunna (Wife); Daniel and Thomas (Sons)
Citizenship:	Swiss (U.S. permanent resident visa)
Soc. Security No.	142-56-7478
Military Service:	Artillery officer in Swiss army

Positions held:

1968 -1971	Privatgymnasium Berne. Teacher of basic chemistry courses (University preparation).
1968–1971	Institute of Forensic Medicine, University of Berne (Prof. E. Lauppi). Assistant supervisor in Laboratory of Toxicological Analysis.
1970–1971	Swiss Association of Laboratory Technicians, Berne. Course organizer and instructor in basic Gas Chromatography.
Oct. 1971 – Jan. 1974	Roche Institute of Molecular Biology, Nutley NJ. Postdoctoral fellow in Department of Physiological Chemistry (Dr. S. Udenfriend).
Feb.–Aug. 1974	University of California Medical Center, San Francisco. Research fellow in Hormone Research Laboratory (Dr. C. H. Li).
Oct. 1974 – April 1978	Merrell International Research Center, Strasbourg, France. Senior Scientist, Section of Experimental Therapeutics.
May 1978 – Jan. 1979	Senior Research Associate in Neuroendocrinology. The Salk Institute for Biological Studies, San Diego, CA.

Jan. 1979 –
Jan. 1983 Assistant Research Professor in Neuroendocrinology. The Salk Institute for Biological Studies, San Diego, CA.

Jan. 1983 –
present Associate Research Professor in Neuroendocrinology. The Salk Institute for Biological Studies, San Diego, CA.

Education:

1962 1968 University of Berne
Institute of Chemistry, Switzerland
Diploma (M.S.) in Chemistry (major), Biochemistry and Physics (minor) (June 1968)

1968–1971 University of Berne
Institute of Forensic Medicine/Institute of Chemistry
Ph.D. in Chemistry (June 1971)
Title of doctoral thesis: Disturbances of the intermediary metabolism of rats after hexane inhalation (Lipid, carbohydrate and energy metabolism). Training in Toxicology, Pharmacology.

1971–1973 Roche Institute of Molecular Biology, Nutley NJ, USA.
Postdoctoral training in protein chemistry. Development of new fluorescent methods for amino acids peptides, proteins and other primary amines (Fluorescamine). Development of new automated analytical equipment for amino acid, peptide and protein analysis.

1974 University of California, Medical School, Hormone Research Laboratory, San Francisco, CA
Postdoctoral training in protein sequencing.
Determination of the primary structure of camel prolactin.

Societies:
Endocrine Society.
American Chemical Society.
American Association of the Advancement of Science.
Association of Swiss Chemists.
German Chemical Society.

Editorial Boards:
Analytical Biochemistry (July 1981–).

Honors:
Roche Institute of Molecular Biology - Fellowship.

Major Accomplishments:

At the Roche Institute:
Development of important new methods in amino acid, peptide and protein analysis (Fluorescamine assays for amino acids, peptides, proteins are now standard assays in most laboratories). Invention and development of automatic analytical instrumentation for amino acid, peptide and protein analysis, including a high sensitivity amino acid analyzer (now commercially available), a peptide analyzer, and a fluorescence peptide/protein monitor for HPLC [now being commercialized]).

At the Merrell International Research Center:
Participation in biochemical (analytical), pharmacological and clinical research during development of novel drugs.

a) Development of GABA-Transaminase inhibitors as an attempt to alter brain GABA levels therapeutically (e.g. in Huntington's disease and epilepsy; γ-vinyl-GABA is now highly promising for treatment of epilepsy).
b) Studies on GABA metabolism in brain.
c) Development of inhibitors of ornithine decarboxylase as a means of inhibiting polyamine biosynthesis and cell division.

At the Salk Institute:

- Development of methodology for microisolation of peptides (including novel approaches to peptide extraction, HPLC, elimina-

tion of losses). Isolation and characterization of several neuropeptides was accomplished using only small amounts of tissue. Development of microamino acid analysis as a routine method (less than 50 pmol peptide needed for determining accurate amino acid compositions).
- Isolation of novel peptides involved in the regulation of pituitary growth hormone secretion: Somatostatin-28 and growth hormone releasing-factor (GRF).

Research Interests:
- Isolation and molecular characterization of novel regulatory peptides as a means to study the regulation of physiological function. In addition to several neuropeptides which we have been studying during the last few years, interest includes peptidic factors controlling cell division (growth factors, inhibitors).

- Biosynthesis of neuropeptides (GRF, Neurotensin).

- Development and use of most advanced methodology related to protein chemistry, in the future also including recombinant DNA techniques.

PEPTIDES AND PROTEINS AS MESSENGERS IN THE COMMUNICATION BETWEEN CELLS AND ORGANS

Professor Dr. Peter Böhlen

A fundamental property of living systems is their ability to adjust to environmental changes. In higher organisms where different organs perform various tasks, optimal adjustment to changes requires a system for communication between individual organs. The nervous and the endocrine systems are the two principal mechanisms by which transfer of information is achieved. The nervous system relies on electric signals which travel along nerves between the brain and peripheral organs. In contrast, the endocrine system uses chemical substances for this purpose. These chemical messengers are secreted by endocrine glands and reach their target organs via the bloodstream.

The involvement of polypeptides as chemical messengers, or hormones, in endocrine regulation systems has long been known. Many hormonal regulatory systems have been well studied; much detail is known but it is also evident that still more needs to be learned before a thorough understanding of all important aspects is possible. The present review is not intended to summarize the vast knowledge which has been accumulated on the various polypeptide hormones and their involvement in vital body functions. Instead, the focus will be on a new development in endocrine research: the recent discovery of a large number of novel biologically active polypeptides with potentially hormonal character, now generally termed regulatory peptides. This development has come as a surprise even to those of us who have been involved in the discovery of novel peptides. While the question as to the biological significance of most of these peptides remains to be answered, the sudden appearance of a multitude of peptides is now already beginning to change our views of the endocrine regulatory system. In reviewing this development and the general concepts emerging from it I hope to be able to demonstrate how new looks at the old concept of intercellular communication via

polypeptides may shape a better understanding of physiological function with all the implications of possible future benefits in the therapy of diseases.

The discovery of new peptide hormones

Traditionally, the discovery of a new peptide hormone has been the consequence of a physiological observation, usually derived from interference with a vital regulatory system in an experimental animal. For example, the presence of hormones in the pituitary or the pancreas was demonstrated by showing that removal of these glands resulted in very deleterious effects to the animal which could be overcome or prevented by giving the animals extracts from the excised glands. Many of the classical polypeptide hormones (insulin, corticotropin, growth hormone, etc.) in the major endocrine secretory glands have been discovered using such physiological probing. These techniques have also provided evidence for the existence of many additional potential regulatory peptides with suspected functions ranging from sleep and reproduction to the immune response. There was little doubt that the isolation and elucidation of the chemical nature of those substances could provide answers to important questions about the mechanisms of physiological regulation.

To this day such reasoning provides one of the most powerful rationales used in the search for novel hormones. The following example may illustrate this further. Much is known about the regulation of blood pressure by the nervous system and by endocrine pathways. Yet clearly, the complex mechanisms of blood pressure control are not well understood. Better knowledge of these mechanisms is of utmost importance, both from biological and the clinical viewpoints. Malfunction of this regulatory system is a frequent cause of hypertension, one of the most prevalent diseases for which ways of prevention and better therapy must be found. Evidence from many lines of research has provided clues on how a better understanding of the problem of blood pressure regulation might be arrived at. Some of the more intriguing findings suggest that an additional endocrine mechanism may be involved: there is evidence that unknown substances, believed to be peptides and existing in the blood (more recently such peptides were also found in the heart) possess natri-

uretic activity, i.e. they promote the excretion of sodium ions through the kidney (1). Since the sodium concentration in the blood may influence blood pressure, it is conceivable that the natriuretic factor participates in its control. The proper investigation of this hypothesis requires that large amounts of substance are available for biological experimentation. Because sufficient amounts cannot be obtained from natural sources, it is necessary to first isolate the natriuretic factor, determine its chemical structure and, with that information make the synthetic replicate of the natural substance in unlimited quantities. Only then the experiments can be performed which ultimately will determine the physiological significance of the natriuretic factor. If shown to be physiologically relevant, the peptide or structurally related active substances can also be evaluated for therapeutic use.

However, despite the attractiveness of such rationalizing, the number of polypeptide hormones with known structures remained small until the early 1970s. The hormones whose structures were elucidated were those which occured in large quantities thus permitting their isolation and characterization with the relatively inefficient methodology then available. Other regulatory peptides, obscured by our inability to define their chemical nature, remained hypothetical. It was realized that the majority of these hormone candidates occur in minute quantities in animal tissue and that their isolation and characterization would therefore be difficult. The structure of the natriuretic hormone is still not known although efforts to elucidate it are currently ongoing (2).

This situation has dramatically changed during the past decade. Today, a large number of biologically active peptides with potential roles in the regulation of many physiological functions is known (Table 1). Moreover, it is highly probable that still more novel bioactive peptides will be found and characterized in the near future. The new information derived from the discovery of those peptides may change our views of the endocrine regulatory system profoundly.

Two independent methodological advances were largely responsible for this development. First, the discovery of new peptides was greatly helped by the development of refined tools for physiological experimentation. A major disadvantage of many older biological tests in animals was their insensitivity to substances that cause only subtle

TABLE 1:

MAMMALIAN REGULATORY POLYPEPTIDES WITH KNOWN CHEMICAL STRUCTURE

	Principal Location	*Known or Suspected Function*
*Adrenocorticotropin (ACTH)	pituitary	steroidogenesis, stress
*Melanotropin(s) (MSH)	pituitary, brain	NP, pigmentation?
*Growth hormone (GH)	pituitary	growth control, metabolism
*Prolactin (PRL)	pituitary	lactation
*Thyroid stimulating hormone (TSH)	pituitary	metabolism
*Luteinizing hormone (LH)	pituitary	reproduction
*Follicle stimulating hormone (FSH)	pituitary	reproduction
*Oxytocin	pituitary	milk ejection
*Vasopressin	pituitary	water resorption, memory?
Vasotocin	pituitary, pineal	NP, reproduction?
Endorphin(s)	pituitary, brain	NP, pain
β-cell tropin	pituitary	control of insulin release?
Lipotropin(s)	pituitary	?
TSH releasing factor (TRF)	hypothalamus	NP, control of TSH, prolactin
LH/FSH releasing factor (LRF)	hypothalamus	NP, control of FSH/LH
Somatostatin(s)	hypothalamus, brain, pancreas, gut, etc.	NP, inhibition of hormone release, many others
Corticotropin-releasing factor (CRF)	hypothalamus	NP, control of ACTH, endorphin
GH-releasing factor (GRF)	hypothalamus	NP, control of growth hormone
Physalaemin	brain	NP
Enkephalin(s)	brain, adrenal	NP
Dynorphins(s)	brain	NP
Neoendorphins(s)	brain	NP
Metorphamide	brain	NP
Rimorphin	brain	NP
Bombesin	brain, gut	NP
Calcitonin gene-related peptide	brain	NP, blood pressure?
Head activating factor	brain, gut	NP
Sleep-inducing peptide	brain	NP, sleep?
Neuropeptide Y	brain	NP

	Principal Location	*Known or Suspected Function*
Substance P	brain, gut	NP, pain
Neurotensin	brain, gut	NP
Vasoactive intestinal peptide (VIP)	brain, gut	NP
Cholecystokinin(s)	brain, gut	NP, satiety?
Carnosine	brain	NP
Homocarnosine	brain	NP
Scotophobin	brain	memory?
Peptide HI	brain, gut	?
*Parathyrin	thyroid	calcium metabolism
*Calcitonin	thyroid	bone formation
*Insulin	pancreas	carbohydrate metabolism
*Glucagon	pancreas	carbohydrate metabolism
Pancreatic polypeptide	pancreas	pancreatic exocrine secretion
*Gastrin(s)	stomach	control of acid production in stomach
*Secretin	gut	control of intestinal pH
Motilin	gut	control of gastric motility
Gastric inhibitory peptide	gut	?
Gastrin releasing peptide	gut	?
Peptide YY	gut	insulin release?
Thymosin(s)	thymus	immune response
Thymopoietin	thymus	immune response
Tuftsin	circulation	immune response
Peptide FTS	circulation	immune response
*Angiotensin(s)	circulation	blood pressure
*Bradykinin(s)	circulation	inflammation
Relaxin	ovary	widening of birth channel
Epidermal growth factor (EGF)	submaxillary gland	tissue repair?
Insulin-like growth factor (IGF)	liver?	growth, metabolism
Nerve growth factor (NGF)	submaxillary gland	maintenance of neurons
T-cell growth factor (TCGF)	lymphocytes	immune response
Platelet-derived growth factor (PDGF)	platelets	?
Transforming growth factor(s)	many normal and neoplastic tissues	?
Fibroblast growth factor (FGF)	brain	?

NP = Neuropeptide; * = Classical peptide hormones

disturbance of the observable, normal state. Minor changes may be compensated by the animal or tolerated without adverse effects or take a long time to become discernible. More advanced tests (called bioassays) are now known. Many of them rely on the measurement of a biological effect in vitro, i.e. they are conducted in a test tube using cultured cells or parts of organs as targets for substances to be investigated. Such tests are often exquisitely sensitive and more specific and therefore ideally suited for the assay of peptides available only in very small quantities (it may typically take one thousand times less material to see an effect of a peptide in an in vitro assay as opposed to an animal test). The second and most important factor for the discovery of new bioactive peptides was undoubtedly the development of highly efficient chemical methodology for the isolation and characterization of peptides. In the past many isolation projects have failed because it was not fully realized in what exceedingly low amounts hormones can occur in tissue. With the methodology available until the early 1970s it was necessary, for example, to extract millions of hypothalami in order to obtain sufficient quantities of pure material for the elucidation of the structures of the first brain peptides, thyrotropin releasing factor (TRF), gonadotropin releasing factor (LRF), and growth hormone release inhibiting factor (somatostatin). During the last ten years new analytical techniques have been developed which greatly increase the efficiency of peptide isolation and characterization. In the early 1970s novel fluorescence methods for the high-sensitivity detection of polypeptides were introduced (3). With these techniques much smaller quantities of material can be detected than was previously possible. The most outstanding methodological advance came when reverse-phase high performance liquid chromatography (HPLC) was developed and successfully applied to the separation of peptides (4). While the isolation of a peptide hormone with conventional methodology could require very long times (months to years) and very large quantities of tissues (thousands to hundreds of thousands of individual organs), HPLC, combined with highly sensitive detection methods made it possible to achieve the same result in a short time (weeks) and with far less tissue (gram amounts). Finally, improved micromethodology for the structural analysis of polypeptides, i.e. amino acid analysis with fluorescence detection (5) and automatic sequence determina-

tion with the gas-liquid phase sequencer (6) was developed. Only with these new analytical methods was it possible to take advantage of the microisolation capabilities of HPLC, i.e. to isolate a small quantity of a peptide and then to be able to determine its chemical structure with just that amount.

New peptides – new hormones?

For any newly discovered peptide the question must be asked whether it truly represents a hormone. By definition, a hormone transfers information between cells for the purpose of regulating a body function. The possibility exists that a biological activity of a peptide as detected in a bioassay is entirely artefactual. Furthermore, a peptide frequently has more than one biological activity which may confuse the biologist in search of the true function. Therefore a newly discovered bioactive peptide must be carefully studied before it can be called a hormone. However, establishing the hormonal status of a new peptide is often difficult. In fact, for most of the new peptides we do not yet know a precise physiological function. Once the structure of a peptide is known, it may still take years of research before its role in the body is discovered; years of gathering detailed evidence, of separating fact from artifact, of integrating old and new data, and sometimes of waiting for the lucky breakthrough provided by a seemingly unrelated observation.

The story of the unraveling of the basic mechanisms by which the body controls its growth illustrates well how a biological puzzle is slowly assembled. It also documents the importance of characterizing novel hormonal peptides as a principal means to gain a better understanding of complex bodily functions. The involvement of the pituitary gland in the control of body growth was discovered in 1922 by Evans & Long. The chemical substance in the pituitary which is responsible for this activity, growth hormone, was isolated in 1944 and the structure of the protein determined in 1966 by C.H. Li and collaborators. Much has been learned about the complex actions of growth hormone on both general metabolic processes and those directly related to growth. Both impaired or excess growth hormone secretion are causes of growth disturbances such as dwarfism or gigantism in children and acromegaly in adults. Until recently very little knowledge existed as to how the body regulates the concentra-

tion of growth hormone in the blood and maintains the balance between the deleterious consequences of hormone over- and undersupply. Likewise, it was not known how the hormone, being itself devoid of stimulatory activity on cell division and multiplication, stimulates bone growth.

Strong evidence pointed towards the brain and particularly the hypothalamus as having a role in this regulatory mechanism. In 1948 Harris had proposed that the secretion of pituitary hormones is regulated by the hypothalamus via endocrine pathways (7). More precisely, the existence of a hypothalamic peptide was postulated which can be released and act upon the pituitary to stimulate growth hormone secretion. Physiological evidence for the growth hormone releasing factor (GRF) was at hand but the isolation of the factor remained unsuccessful despite many attempts. Instead, the quest for GRF led to the surprising discovery of two hypothalamic growth hormone release inhibiting factors – somatostatin-14 and somatostatin-28 (8, 9). Was growth hormone secretion controlled by an inhibitory rather than a stimulatory messenger? A variety of tests established a physiologic role for somatostatin in growth hormone control but also showed that the action of an inhibitory substance alone was not sufficient to account for the available experimental data. The search for GRF continued but turned out to be particularly difficult, even with the best methodology available in 1981. Again, an unexpected discovery provided the insight to solve the GRF problem. This time the clue came from clinicians who had observed that in rare cases patients with lung or pancreas tumors also developed acromegaly from excess growth hormone secretion. Normally, acromegaly is caused by pituitary tumors but in these patients pituitary abnormality was ruled out. The existence of growth hormone releasing substances in these tumors was suspected. In 1981, while working on the isolation of rat hypothalamic GRF, our laboratory received small pieces of lung and pancreas tumors that had caused acromegaly. GRF-like substances were isolated from both tumor pieces. After their partial structural characterization, we quickly realized the close similarity of these substances with authentic hypothalamic GRF. With more material from yet another acromegalic patient with a pancreas tumor we succeeded in isolating sufficient quantities to determine the structure of pancreas GRF (10). The

identity with hypothalamic GRF was established by partial characterization of human hypothalamic GRF (11) and confirmed finally by the isolation and characterization of the closely related porcine hypothalamic GRF (12). The latter is so close in structure to pancreas GRF that there is no doubt that the tumor material corresponds to the normal hypothalamic peptide.

The question as to how skeletal growth is stimulated by growth hormone was also answered through the discovery of a novel peptide. For some time it was noticed that growth hormone acts upon the liver to release a factor, somatomedin, that promotes bone growth (13). The question, whether this factor is part of the mechanism which controls skeletal growth, could not be answered because it proved to be very difficult to isolate somatomedin. Again, a seemingly unrelated discovery provided the key for the solution of the problem. The groups of Froesch and Humbel in Zurich had described and eventually characterized two peptides which are insulin-like in structure and have potent growth promoting activity (14). Further investigation of their function showed that these insulin-like growth factors (IGF) were growth hormone-dependent and had all the biological properties of the somatomedins. Careful comparison of IGF and somatomedins established that they were, indeed, identical.

The discovery of somatostatins, somatomedins and GRF and the demonstration of their physiological functions formed the basis for postulating a regulatory mechanism for the control of growth hormone secretion (Fig. 1). GRF induces the release of growth hormone which then stimulates the release of somatomedin (probably from the liver). Somatomedin induces chondrocytes to proliferate, a process necessary for the formation of bone matrix and skeletal growth. A second function of somatomedins is to feed back to the pituitary the information that enough of this peptide is present for function and further secretion of growth hormone is unnecessary. We know that somatomedin does this by counteracting the effect of GRF on the pituitary. The hypothalamus exerts additional inhibitory control on growth hormone secretion through somatostatin; the precise function of this mechanism is still unclear.

This view of growth hormone regulation is certainly incomplete. There is evidence, for example, that the release of GRF from the

hypothalamus is controlled by higher brain centers. Likewise, the involvement of thyroid hormones and sex steroids in growth processes is well known. However, very little is known about the integration of the action of these hormones into the overall regulation of growth processes. The role of the growth hormone regulatory system also needs to be better understood in the adult organism, where skeletal growth no longer occurs.

Diversity in peptide hormone regulatory systems

Biologists are now faced with the existence of a large and rapidly increasing number of bioactive peptides for most of which a physiological function is suspected but not proven. It is tempting to speculate that each physiological function is regulated by a set of specific peptides. If this were true then a very large number of regulatory peptides would still remain to be discovered and we would have to

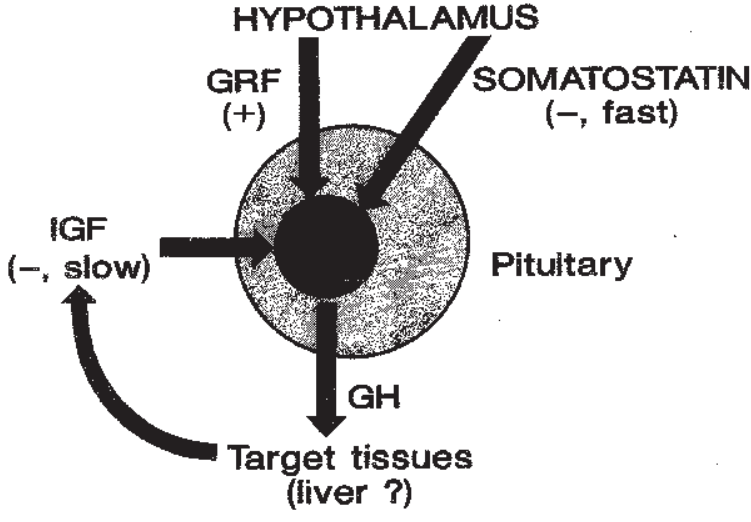


Fig. 1
Physiological regulation of growth hormone secretion.
GRF: growth hormone releasing factor; GH: growth hormone; IGF: insulin-like growth factor; +: stimulatory effect; –: inhibitory effect.

find ways of identifying them. In this context it is of interest to look at the way a cell synthesizes these regulatory peptides. Recent insight into these biosynthetic mechanisms suggests that the cell uses some rather intriguing methods to generate structural and functional diversity among peptides. The biosynthesis of a peptide is a complex process which includes (a) the reading of information from a gene and its duplication as messenger RNA (transcription), (b) the manufacturing of a protein, often called precursor protein using the messenger RNA duplicate (translation of the messenger RNA), and (c) the degradation and/or modification of the inactive precursor protein into pieces one of which is the biologically active polypeptide (co- and post-translational processing).

To synthesize a large protein for the purpose of generating only a small usable fragment (the bioactive peptide) seems to be wasteful. It appears, however, that rather than wasting the excess portion of a precursor protein, nature can actually take advantage of it. This has become apparent for the first time when the structure of the precursor for the well-known hormone, adrenocorticotropin was elucidated and it was found that the sequences of other suspected regulatory peptides, β-endorphin and β-lipotropin, was contained in it (15). In other words a precursor protein may serve for the production of more than one biologically active peptide. The same phenomenon has been observed with other precursor proteins and it is now believed that it could represent a fairly general mechanism for the biosynthesis of diverse regulatory peptides. There is also increasing evidence indicating that this mechanism provides the organism with a means to use the same precursor protein to generate different bioactive peptides in different organs, as long as the availability of the proper processing enzymes (the enzymes which cut a precursor protein into smaller peptides) within a given organ is controlled. Furthermore, the existence of novel regulatory peptides may be predicted by analyzing the structures of precursor proteins for the presence of enzymatic cleavage sites (typical structural features known to be cleaved by processing enzymes). It is remarkable that several peptides predicted using this rationale have now already been isolated and found to possess biological activity (16, 17).

The complex pattern of peptides that can be generated by post-translational processing of a single precursor protein is illustrated in

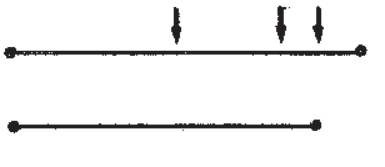
Table 2. This example shows clearly how differential processing can lead to a multitude of peptides. While some of these peptides are structurally and functionally related (e.g. somatostatin-14 and -28) others bear no relation (e.g. somatostatin-14 and somatostatin-28 [1–12]). Several of these peptides have been isolated and characterized but others are hypothetical and their presence in one or the other tissue remains to be established. Likewise, a physiological function is known for only a few of these peptides. The others may possess as yet unknown activities, or may be useless waste products arising from the generation of other functional peptides.

Peptide diversity is also generated by posttranslation modification of precursor proteins or bioactive peptides. Such modification includes, for example, glycosylation, phosphorylation, and acetylation, i.e. the attachment of carbohydrate, phosphate, acetate residues, etc. to certain structures within a polypeptide. Many peptide hormones occur in several modified forms; corticotropin-like intermediary lobe peptide exists in the rat pituitary in eight different forms, varying in their degree of glycosylation and phosphorylation (18). Very little is known about the function of such modified peptides.

Recently an entirely new mechanism has been discovered which involves altered RNA processing as a means to produce peptide diversity. There are two ways how the primary gene transcript for the peptide hormone calcitonin can be processed, one leading to a messenger RNA that translates into calcitonin and the other to a messenger RNA coding for a peptide previously not known to exist (19). A search for this new peptide lead to its isolation within a short time. Interestingly, the new peptide, termed calcitonin gene-related peptide, is devoid of calcitonin-like bioactivity and does not exist in the thyroid gland, the host tissue of calcitonin. Rather, it is found in the brain and in nerves innervating blood vessels. Preliminary studies indicate that the peptide is a powerful blood pressure-reducing agent. The example is unique so far but may perhaps represent yet another more general mechanism for the creation of peptide hormone diversity. As with the "one precursor – multiple hormones" concept the significance may be that this mechanism allows the organism to use the same basic structures, after tissue-specific modification, to regulate different physiological functions in different organs.

TABLE 2:

PEPTIDES DERIVED FROM PRO-SOMATOSTATIN (PRO-SS)



Peptide	*Location*	*Activity*
Pro-SS 1–92**	hypothalamus, pancreas, brain gastrointestinal tract	precursor
Pro-SS 1–76**	brain	?
Pro-SS 1–64***	?	?
Pro-SS 1–32***	?	?
Pro-SS 33–92**	brain	?
Pro-SS 33–76*	brain	?
Pro-SS 33–64***	?	?
Somatostatin-28* (Pro-SS 65–92)	hypothalamus, pancreas lower gastrointestinal tract	many activities
SS28 (1-12)* (Pro-SS 65–76)	hypothalamus, pancreas, cortex	neuromodulator (?)
Somatostatin-14 (Pro-SS 79–92)*	hypothalamus, pancreas upper gastrointestinal tract	many activities

Arrows indicate known enzymatic cleavage sites of prosomatostatin. Peptides denominated with asterisks: *fully characterized; **identified, but not fully characterized; ***hypothetical, not yet found in tissues.

Functional diversity in peptide regulatory systems also derives from multiple activities that can be associated with the bioactive peptides. Table 3 summarizes the many biological activities of somatostatin. Depending on the target location somatostatin has entirely different activities and most likely even physiological functions. Somatostatin is no exception; for most other regulatory peptides multiple activities are known.

Finally, it should be mentioned that more and more regulatory peptides are found which do not fit the classical definition of a hormone, i.e. the chemical substance that transmits information between organs by means of the bloodstream. In addition to the classical "endocrine" mechanism the "paracrine" and "autocrine" systems are now known. Both are similar to endocrine pathways as they rely on chemicals for the exchange of information but do not use the bloodstream as a means of message transportation. The paracrine system is characterized by an information transfer between two different types of cells via the intercellular fluid space which requires that the communicating cells are in relatively close proximity. Numerous examples for this type of mechanism can be cited; it should suffice to mention here that many brain peptides (neuropeptides) are thought to act in this manner. It is known that many peptides can act through endocrine and paracrine mechanisms depending on their function at a particular location. The third type, the "autocrine" system resembles paracrine secretion. Communicating cells are located closely together but hormonal messengers act upon the same cell type from which they are released. Autocrine regulation systems seem to be associated with certain growth factors. Examples are still few and physiological significance of this mechanism is unclear.

TABLE 3:

BIOLOGICAL ACTIONS AND PHYSIOLOGICAL FUNCTIONS OF SOMATOSTATIN

Actions:

inhibition of endocrine secretion:	growth hormone thyrotropin insulin glucagon gastrin, secretin and other gastrointestinal hormones
inhibition of exocrine secretion:	pancreatic enzyme and electrolyte secretion, gastric acid secretion
inhibition of other functions:	nutrient absorption in gut blood flow (intestine) water transport (kidney) platelet aggregation electrical activity of brain neurotransmitter release

Physiological functions:

regulation of growth hormone secretion
regulation of carbohydrate utilization
regulation of intestinal function (?)
neuromodulator

SUMMARY AND OUTLOOK

Technological advances in the 1970s lead to the discovery of a large number of new bioactive peptides. Although little definitive evidence is yet available, many of these peptides must be considered as candidates for having a physiological function. In view of the multitude of peptides, and the rather bewildering arrays of activities, locations and targets of many of them, more physiological research will be necessary before a reasonable understanding of new regulatory processes is possible. Despite these present limitations, new concepts of endocrine function and regulation have already been established and more are beginning to emerge.

The discovery of the first brain hormones (TRF, LRF, Somatostatin) by the groups of Guillemin and Schally in the early 1970s represents the most outstanding example of how the discovery of new peptides can lead to new biological concepts. This achievement established the discipline of neuroendocrinology which proposes that many body functions are controlled by the brain via endocrine pathways (20). The view is now increasingly accepted that peptides play a far greater role in controlling body functions than was thought even a short time ago. Circumstantial evidence from many areas of physiological research is rapidly accumulating which strongly supports this notion (Table 4) and will probably result in the discovery of many additional regulatory peptides in the near future. It is conceivable that regulatory peptides are involved in the control of most if not all bodily functions.

Regulatory peptides, even though not understood in detail, are beginning to shape new insights into the functioning of some of the most complex and poorly understood regulatory systems of our body. One of the most difficult problems in biology is to understand brain function, how the enormously complex network of neurons inputs, processes, organizes and finally outputs a steady flow of information. A large number of the presently known bioactive peptides belongs to the class of neuropeptides, i.e. they are present in some structure of the brain. Neuroscientists haven been puzzled as to why their number is so high and what their function is. It is now known that neuropeptides have the ability to alter (modulate) the transmission of signals along nerves and it has been postulated that

TABLE 4:

EXAMPLES OF HYPOTHETICAL PEPTIDES THOUGHT TO BE INVOLVED IN PHYSIOLOGICAL FUNCTION

natriuretic hormone	blood pressure regulation
insulin releasing factor	carbohydrate homeostasis
glucagon releasing factor	carbohydrate homeostasis
lymphokines (B-cell growth factor, interleukin-1, macrophage activating factor, etc.)	immune response
immunosuppressive peptides	immune response
inhibin	reproductive function
sleep factors	sleep
angiogenesis factor	blood vessel formation
endothelial cell growth factors	blood vessel formation
other growth factors	cell growth/division (e. g. wound healing)
chalones, growth inhibitors	cell growth/division
erytrhopoietin	blood cell formation
granulopoietin	blood cell formation
colony stimulating factor	blood cell formation
osteoblast activating factor	bone formation
bone morphogenetic protein	cell differentiation
transforming gene products	tumorigenesis?

the presence of a large number of neuropeptides could influence the circuitry of the brain in countless ways. In addition to the anatomical organization of the nervous system, the neuropeptide system may represent a principal mechanism contributing to the brain's enormous capability to process information and organize its flow.

Similarly, with the discovery of regulatory peptides that can modulate the immune response new views of the functioning of the immune systems are beginning to emerge. Only a few of these polypeptides, now termed lymphokines, have so far been found (e.g. T-cell growth factor, B-cell growth factor, macrophage activating factor, interferon) and most of them are still very poorly characterized. However, it is already clear that these regulatory polypeptides have important functions in the complex regulation of the immune response. The discovery of these peptides suggests that the immune system is endocrine in nature in many respects. Only a short time ago, this would have been a rather unacceptable view.

Finally, the possibility must be considered that biologically active peptides will be used as therapeutic agents in the treatment of a variety of diseases. Although peptides have been used as therapeutics (insulin, etc.) in the past, they have, with few exceptions not gained wide-spread acceptance in human medicine. This situation is likely to change. The rapidly growing knowledge on bioactive peptides is a unique and virtually untapped resource for the development of new therapeutic and diagnostic agents and procedures. The exploitation of this resource will not come easy for a variety of reasons but efforts in this direction are expected to pay off in the long run. As the difficulties faced by the medicinal chemist in developing novel drugs by traditional approaches are ever increasing, some early successes of biotechnology (e.g. interferon) indicate that the use of nature's own tools to combat disease may become a viable alternative.

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ÉLOGE DU DOCTEUR CLAES B. WOLLHEIM

Professeur A.F. Muller

C'est la seconde fois que votre Fondation me fait le plaisir et l'honneur de présenter un titulaire du Prix Max Cloëtta. C'est là un des avantages de diriger un Département de médecine interne, celui de pouvoir participer au succès des collaborateurs dans différentes divisions du même Département. Le Dr Claes Wollheim, que je vous présente aujourd'hui, est né il y a presque jour pour jour 40 ans à Lund, en Suède, où son père, alors Directeur du Département de Pharmacologie, avait trouvé refuge pendant la sombre époque du nazisme en Allemagne. Puisque le domaine de la pathogénèse de l'hypertension a été l'un des domaines préférés de mon activité scientifique, je me plais à rappeler aujourd'hui l'importance de la contribution du père de notre lauréat à la compréhension de la pathogénèse de l'hypertension et j'ai vu avec plaisir que, peu avant la mort de votre père, vous avez, Claes Wollheim, collaboré avec lui dans la rédaction du dernier travail scientifique qui lui a été donné de publier.

Vous avez été vous-même élevé en Suède où vous avez obtenu votre titre de Docteur en Médecine en 1970. C'est en 1971 déjà que, sur le conseil de votre mentor et ami, le Professeur Helmreich de l'Université de Würzburg, vous êtes venu à Genève, à l'Institut de Biochimie clinique, où vous attirait la recherche biomédicale dans le domaine du métabolisme et de sa régulation. Vous hésitiez encore sur un avenir partagé entre la clinique et la recherche fondamentale ou un choix résolu et à long terme pour la recherche. Votre décision en faveur de la seconde option a été facilitée par la fascination toujours plus grande que vous avez ressentie pour les relations entre structure et fonction au niveau de la membrane plasmatique et également au niveau des organelles cellulaires, et tout particulièrement pour le rôle du calcium dans l'organisation et le fonctionnement de la cellule. Votre décision a également été catalysée par le contact avec les chercheurs et collaborateurs venus de l'étranger, dont beaucoup venaient passer six mois, une ou même deux années sabbatiques à l'Institut de Biochimie clinique, institut spécialisé dans la coordination des activi-

tés d'un groupe international de chercheurs plus jeunes ou un peu moins jeunes. C'est ainsi que vous avez particulièrement profité de l'association étroite qui s'est établie entre vous et le Professeur Goeffrey Sharp, maintenant Directeur de la Section des Sciences biologiques de la Cornell University à Ithaca. A l'époque, ce dernier était actif au Département de Médecine du Massachusetts General Hospital et c'est avec lui qu'un échange constant d'idées, de méthodes et de visites, a beaucoup contribué aux dialogues intensifs nécessaires à toute contribution scientifique originale. Vous avez vous-même contribué de façon très importante à maintenir dans l'Institut de Biochimie clinique, qui fait partie du Département de Médecine, cet esprit de curiosité partagée et de compréhension mutuelle qui est à la base de toute collaboration d'un groupe scientifique guidé par l'échange des connaissances, l'intérêt pour l'inconnu, l'imagination créatrice et l'exactitude critique, toutes des qualités nécessaires et requises pour la réussite d'une équipe d'excellence. Si tout cela vous avez pu le faire sans passer de longs séjours à l'étranger, c'est que vous étiez aidé par l'ambiance d'un laboratoire international, mais aussi parce que vous avez œuvré de toutes vos forces afin de maintenir à Genève ce tissu de contacts internationaux dont vous avez et dont nous avons tous absolument besoin. Il est certain que vous avez été soutenu non seulement par le laboratoire où vous avez pu travailler avec les nombreux et proches collaborateurs qui ont été les vôtres, mais aussi par la personne qui vous est la plus proche, l'épouse qui vous aide chaque jour par sa compréhension, sa chaleur et son support efficace, tout comme par le plaisir que vous donnent vos enfants.

Entre 1971 et 1983 vous avez suivi de très près une ligne de recherche dont le but était d'identifier le signal majeur et principal permettant de régler à chaque instant, de minute en minute, la sécrétion de l'insuline par les cellules B des îlots de Langerhans. L'importance probable des ions calcium dans cette régulation a été d'abord soupçonnée vers 1970 lorsque l'appartenance des îlots de Langerhans aux tissus susceptibles de propager biophysiquement une excitation a été soupçonnée à son tour, alors que cette caractéristique avait jusque-là été réservée exclusivement aux différents types de tissus nerveux et musculaires. L'intervention dans ce processus de messagers hormonaux ou seconds, puis finalement et surtout la mesure exacte et novatrice, par l'application de méthodologies entièrement originales, a

permis d'aboutir à une première estimation des relations entre les concentrations intracellulaires extrêmement faibles de calcium et la sécrétion d'hormones. Pour qu'un tel résultat soit obtenu, vous avez eu besoin de toute la patience et la persévérance d'un expérimentateur chevronné; vous avez su utiliser la collaboration et la participation de chercheurs amis et de méthodologies appartenant à d'autres disciplines et vous avez abouti à un résultat dont vous allez nous tracer le principal et pour lequel vos maîtres, vos collègues et vos collaborateurs vous sont reconnaissants.

Pour terminer, je me tourne vers les membres du Conseil de Fondation qui représentent ici même l'héritage du Professeur Max Cloëtta et qui, année après année, sélectionnent des chercheurs qualifiés de notre petit pays pour le Prix Max Cloëtta. Notre profonde reconnaissance leur est acquise. Plus que jamais les universités suisses ont besoin du soutien et de l'encouragement de fondations privées.

LE PRIX CLOËTTA 1985
EST DÉCERNÉ À

MONSIEUR

CLAES B.G.M. WOLLHEIM

DOCTEUR EN MÉDECINE

NÉ EN 1943, PRIVAT-DOCENT ET MAÎTRE D'ENSEIGNEMENT
ET DE RECHERCHE À LA DIVISION DE DIABÉTOLOGIE EXPÉRIMENTALE
ET DE BIOCHIMIE CLINIQUE DU DÉPARTEMENT DE MÉDECINE
DE L'UNIVERSITÉ DE GENÈVE,
POUR SES CONTRIBUTIONS À LA RÉGULATION DE LA SÉCRÉTION
DE L'INSULINE ET D'AUTRE HORMONES PANCRÉATIQUES
ET, EN PARTICULIER, À LA MEILLEURE
COMPRÉHENSION DU RÔLE JOUÉ PAR LE TAUX DE CALCIUM
IONISÉ INTRACELLULAIRE EN TANT QUE MÉDIATEUR
DU CONTRÔLE DE LA SÉCRÉTION INSULINIQUE.

ZURICH, LE 21 NOVEMBRE 1985

POUR LE CONSEIL DE FONDATION:

LE PRÉSIDENT:

LE VICE-PRÉSIDENT:

UN MEMBRE:

PD Dr. Claes B. Wollheim

KURZBIOGRAPHIE VON HERRN PD DR. CLAES B. WOLLHEIM

Geburtsdatum	5. November 1943
Geburtsort	Lund, Schweden
Staatsangehörigkeit	Schwedisch
Zivilstand	Verheiratet, zwei Kinder (geb. 1972/1974)
Schule/Universität	Realgymnasium, Kathedralschule Lund Matura 1962 1963 Medizinische Fakultät, Lund 1970 (Juni) Medizinische Fachprüfung und Doktortitel
Klinische Ausbildung	1968–1969 Vertretungen als Assistent, Abteilung für Infektionskrankheiten des Departements für Innere Medizin, Universität Lund (6 Monate) und Abteilung für Innere Medizin, St. Görans Sjukhus, Stockholm (4 Monate) 1970 6 Monate obligatorischer Distriktsarztdienst, Falkenberg, Schweden 1971 (8 Monate) Assistenzarzt am Departement für Innere Medizin, Universität Lund
Akademische Laufbahn	1971 (September) Wissenschaftlicher Assistent, Institut de Biochimie Clinique, Departement für Innere Medizin, Universität Genf 1972 (Oktober) – 1982 Chargé de Recherche, Institut de Biochimie Clinique, Genf 1979 Privatdozent an der Medizinischen Fakultät, Genf 1982 (Juli) Maître d'enseignement et de recherche, Institut de Biochimie Clinique, Universität Genf

REGULATION OF INSULIN RELEASE BY INTRACELLULAR CALCIUM

P.D., Dr. med. Claes B. Wollheim

Introduction

Insulin is the single most important hormone involved in blood glucose homeostasis. As insulin is extremely potent and some of its effects are very prompt, the fine regulation of its secretion is of paramount importance. Insufficient insulin release leads to various forms of diabetes mellitus associated with high blood glucose, while overproduction of the hormone is encountered in obesity. Tumoral growth of the insulin-producing cell, the β-cell of the endocrine pancreas, results in a lowering of blood glucose. The islets of Langerhans, which are scattered throughout the mammalian pancreas, contain several endocrine cell types of which the β-cell is the most abundant (approximately 70% of the islet cells). Insulin, like other peptide hormones, is stored in the β-cell in membrane-enclosed secretory granules. Activation of the secretory mechanism involves the recognition of the stimulus by the β-cell, the generation of a signal or signals resulting in movement of the secretory granules towards the plasma membrane and the fusion of the granule and plasma membranes. This membrane-protected extrusion of insulin from the cell is called exocytosis and is similar for a large number of proteins and neurotransmitters stored within granules.

The crucial event in cell activations such as muscle contraction, neurotransmitter release or hormone secretion, is thought to be an increase in the free calcium ion (Ca^{2+}) concentration in the cytoplasma (cytosol) of the cells. Let us first consider this trigger function of Ca^{2+} in the muscle cell.

Some historical aspects

In 1883, one hundred years ago, the British physiologist, Ringer, carried out his classical experiments, demonstrating the role of Calcium in heart muscle contraction (1). Initially he found that water could replace blood in sustaining contraction in an isolated frog heart preparation. Ringer then realised that he had erroneously been using

TABLE 1:

CONCENTRATION OF SOME IONS INSIDE AND OUTSIDE MAMMALIAN CELLS

	Concentration mmol/l H_2O	
Ion	*Inside cell*	*Outside cell*
Na^+	15	150
K^+	130	6
Cl	9	125
Mg^{2+}	1*	1

* This is the concentration of ionized Mg^{2+} which corresponds to 10% of total cellular Mg^{2+}.

tap water rather than distilled water and after analysis of the water, he was able to demonstrate that Calcium was the active principle. At that time Ringer did not distinguish between calcium and ionized Ca^{2+} and it was left for Mc Lean and Hastings to establish firmly that only the ionized, diffusible form of serum (or plasma) calcium is immediately biologically relevant, and to define the conditions for Ca^{2+} measurement in the blood (1a). In 1943, more than half a century later, Kamada and Kinosita, using an isolated muscle preparation of denuded fibres to which they applied various substances, showed that Ca^{2+} was able directly to cause contraction of the muscle cell (2). The classical experiments by Heilbrunn und Wiercinski clearly revealed that Ca^{2+} was the only physiological substance that triggered contraction when microinjected into frog muscle fibres (3). Heilbrunn suggested that calcium was a general intracellular messenger substance which specifically links excitation arising when the nerve impulse reaches the muscle cell membrane to contraction. This concept was later termed excitation-contraction coupling by Sandow (4). The final direct demonstration of the key role of calcium in excitation-contraction coupling was provided by Ashley and Ridgway (5), who more than a decade ago measured changes in the concentration of free cytosolic Ca^{2+} during contraction of amphibian muscle loaded with the Ca^{2+} sensitive photoprotein, aequorin. They found that the intracellular Ca^{2+} concentration rises after depolarisation of the cell membrane but before the increase in muscle tension, a time-course which fits well with the proposesd coupling function of calcium.

Cellular calcium handling

Why is calcium particularly well-suited to exert a coupling or trigger function in the activation of cellular responses? To provide an answer to this question it is necessary to review how the ion is handled by cells (6, 7). The unique properties of the cell membrane are responsible for the differences in ionic composition between the intracellular and extracellular fluid (see Table 1). The cell membrane is practically impermeable to intracellular proteins and other organic anions, which make up most of the intracellular anions. The membrane is moderately permeable to Cl^- and K^+ and less permeable to Na^+ and Ca^{2+}. It should be noted in this context that the cell mem-

brane is much more permeable to water than to ions. There is a potential difference across the cell membrane with the cell interior being negative, relative to the cell exterior. If the ions were subject to only passive movement the cells would be expected to gain Na^+, which moves down its electrochemical gradient, and lose K^+, which moves along its chemical gradient (Table 1). However, the concentration of these positively charged ions (cations) in the cell remains constant because there is active transport of Na^+ out of the cell at the same time as K^+ is taken up. The energy for this ionic movement is derived from the breakdown of the energy rich phosphate bond of adenosine triphosphate (ATP) and the enzyme which pumps Na^+ out of and K^+ into the cell is Na^+/K^+ ATPase, also referred to as the Na^+ pump (Fig. 1).

The concentration of ionized Ca^{2+} in the extracellular fluid of mammals is approximately 1 mM (10^{-3} M). The concentration of ionized Ca^{2+} in the cytosolic compartment of a variety of cells is now known and this calcium, $[Ca^{2+}]_i$ is in the order of 10^{-7} M. There exists thus an inward chemical gradient of Ca^{2+} of 10,000! In addition to this chemical gradient the electrical gradient also favours

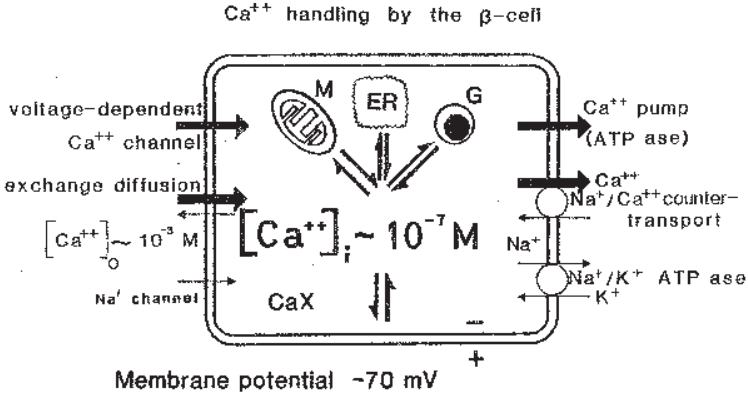


Fig. 1
Calcium handling in the β-cell. Ionized calcium (Ca^{2+}) enters the cell and binds to cytoplasmic proteins (Ca X) or is taken up by endoplasmic reticulum (ER), mitochondria (M) and secretory granules (G). Calcium also binds to the plasma membrane. ATP, adenosine triphosphate.

Ca^{2+} entry into cells (Fig. 1). The Ca^{2+} permeability of the cell membrane is very low thus counteracting the attractive force of the gradients. As in the case for Na^+, passive Ca^{2+} influx can be stimulated severalfold by the gated entry of Ca^{2+} through ion selective channels in the plasma membrane, of which there are both voltage-dependent (Fig. 1) and voltage-independent varieties. The voltage-dependent Ca^{2+} channels open upon depolarisation of the plasma membrane, i.e. when the potential difference across the membrane is decreased. This occurs, for example, during electrical activation of skeletal muscle and heart as well as during neurotransmitter release from nerve endings, or as we shall see, during insulin release from the β-cell.

Two separate processes maintain the intracellular free Ca^{2+} concentration low: 1) the extrusion of Ca^{2+} across the plasma membrane and 2) intracellular binding of Ca^{2+} and uptake of the ion into mitochondria, endoplasmic reticulum and possibly, secretory granules (Fig. 1). The combined activity of these processes, most of which are energy-dependent, results in the extraordinarily low cytosolic Ca^{2+} level.

How did this phenomenon arise? It has been suggested that the need for a low cytosolic free Ca^{2+} concentration was an evolutionary necessity (6). Thus, in the primordial sea, the cell developed a dependency on phosphate bond hydrolysis for its energy requirement. An example of such hydrolysis is the Na^+/K^+ ATPase we have just discussed. Originally, the concentration of Ca^{2+} in the primordial sea was low but rose gradually over eons to reach a level where many cellular phosphate esters would precipitate. It became thus necessary to exclude Ca^{2+} from the intracellular water. By accomplishing this the cell could also take advantage of the Ca^{2+} gradient to transfer information from the cell surface to its interior.

The fine tuning of Ca^{2+} entry is accomplished by brief opening and closing of the voltage-dependent Ca^{2+} channels of excitable tissues such as nerve, muscle and the β-cell. These tissues are characterized by the generation of rhythmical changes of the membrane potential upon electrical or metabolic stimulation. These changes in transmembrane potential can be recorded with intracellular microelectrodes even in such small cells as the β-cell (7). Many tissues, both excitable and non-excitable, are in addition equipped with non-vol-

tage dependent Ca^{2+} channels often activated by the binding of hormones or neurotransmitters to membrane receptors. Thus, the possibility of rapidly changing the ionized Ca^{2+} in the cell cytosol combined with the property of Ca^{2+} to bind to certain cellular proteins, make Ca^{2+} particularly suitable to carry out the coupling function in stimulus-response coupling.

Calcium in stimulus-secretion coupling in the β-cell

We have already considered one example of stimulus-response coupling, namely excitation-contraction coupling in the muscle. In 1961 Douglas and Ruin proposed the concept of stimulus-secretion coupling for adrenalin release from the adrenal medulla and their experiments emphasized the role of calcium ions in this process (8). The concept was later extended to insulin release from the β-cell and we shall now consider some of the evidence.

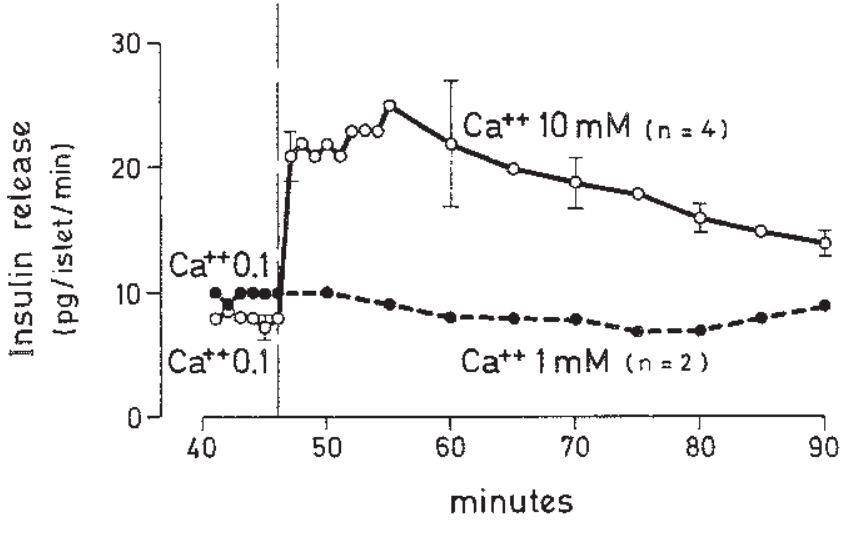


Fig. 2
Calcium-stimulated insulin release. Isolated rat pancreatic islets were first perifused with buffer containing 0.1 mM Ca^{2+}. The Ca^{2+} concentration was then raised to 10 mM (solid line) or to 1 mM (dotted line). See reference 7.

One of the questions to be answered was whether Ca^{2+} itself is able to elicit insulin release. As we have seen, Ca^{2+} permeability of the plasma membrane in the resting state is low. Small changes of the extracellular Ca^{2+} concentration around the physiological level, therefore, are ineffective. However, when the cells are pretreated with a low Ca^{2+} concentration which depletes certain binding sites for Ca^{2+} in the plasma membrane, subsequent exposure to an unphysiologically high Ca^{2+} concentration stimulates insulin release in the absence of other stimuli (Figure 2). Similar results were obtained when Ca^{2+} entry into the cells was facilitated by the use of Ca^{2+} ionophores. These lipophilic substances bind Ca^{2+} and transport the ion across the plasma membrane down the chemical gradient for Ca^{2+}. However, excessive Ca^{2+} entry has adverse effects on cell function as seen from experiments with the Ca^{2+} ionophore A23187, in that both insulin release from β-cells and glucagon release from the neighbouring cell in the pancreatic islet, the α-cell, can be stimulated by low and inhibited by high ionophore concentrations (9, 10).

It has been known since the late 1960s that the presence of extracellular Ca^{2+} is necessary for the stimulation of insulin release by the main physiological activators of the release process, glucose and certain amino acids (reviewed in 7). This suggests that these substances depend on the stimulation of Ca^{2+} uptake for their action. By incubating isolated islets of Langerhans in the presence of radioactive $^{45}Ca^{2+}$, calcium uptake can be measured. The results of such experiments where insulin release was assessed simultaneously with Ca^{2+} uptake, are shown in Fig. 3. It can be seen that a high glucose concentration stimulates both Ca^{2+} uptake and insulin release.

Is the increase in Ca^{2+} uptake due to opening of voltage-dependent Ca^{2+} channels? Two lines of evidence suggest this: 1) glucose depolarizes the β-cell membrane and induces rhythmical electrical activity with action-potential-like spikes, which are believed to represent inward Ca^{2+} current (7); 2) blockers of voltage-dependent Ca^{2+} channels such as verapamil (11) and cobalt ions (12) inhibit both glucose-stimulated Ca^{2+} uptake and insulin release. In Fig. 4 is shown the effect of two cobalt concentrations. The dynamics of insulin release and $^{45}Ca^{2+}$ efflux from islets preloaded for two days with the isotope are depicted. A rapid and sustained increase of the

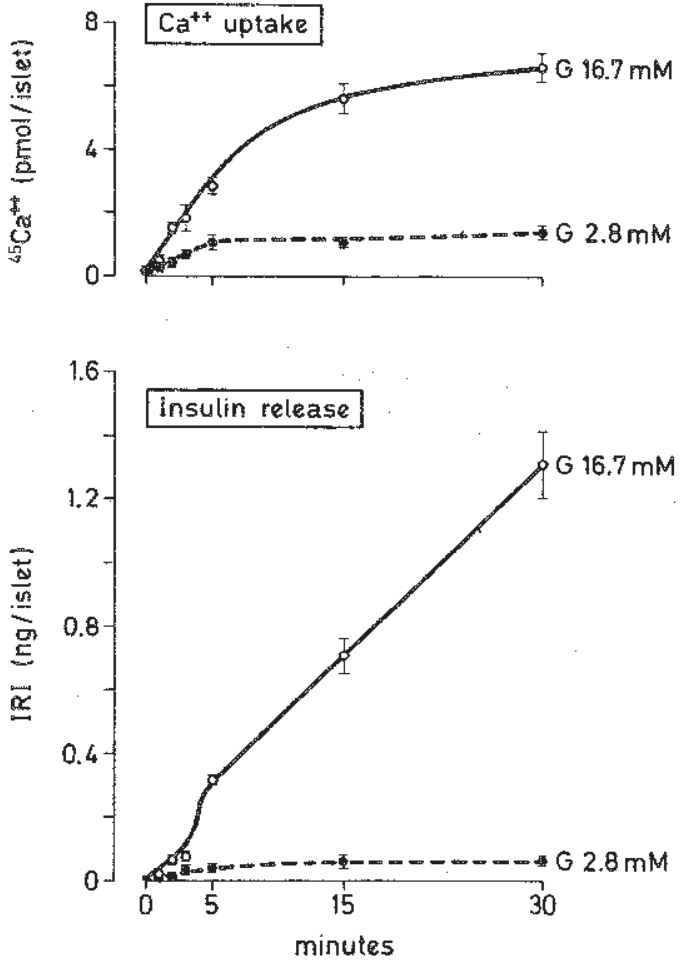


Fig. 3
Calcium uptake and insulin release stimulated by glucose in isolated rat islets. Both variables were measured simultaneously by incubating the islets with $^{45}Ca^{2+}$ and (^{3}H) sucrose, serving as a marker of the extracellular space. G, glucose. See references 7 and 10.

glucose concentration perifusing the islet cells results in the typical biphasic pattern of stimulated insulin release (Fig. 4, lower panel), which is also observed *in vivo*, when insulin is measured directly at the exit of the pancreas, i.e. in the portal vein. The release is accompanied by a similarly biphasic increase in the efflux of radioactive Ca^{2+} (Fig. 4, upper panel). In view of the striking parallelism in the kinetics of insulin release and $^{45}Ca^{2+}$ efflux, it has been proposed that the changes in $^{45}Ca^{2+}$ efflux reflect changes in the concentration of cytosolic Ca^{2+} (7). The increase in $^{45}Ca^{2+}$ efflux induced by glucose is not due to passive $^{45}Ca^{2+}$ release with insulin during exocytosis, as the two phenomena can be completely dissociated. This is also seen in Fig. 4 where the lower cobalt concentration inhibited insulin release considerably more than $^{45}Ca^{2+}$ efflux. In the presence of a high cobalt concentration, glucose is no longer able to stimulate insulin release, thus suggesting that Ca^{2+} entry through voltage-dependent Ca^{2+} channels is essential for the stimulation of insulin release.

The mechanism by which glucose depolarizes the β-cell membrane is unknown, but the sugar hexose needs to be metabolized by the cells. Thus, the effects of glucose on membrane potential, Ca^{2+} fluxes and insulin release are reproduced by the triose, glyceraldehyde. Glyceraldehyde phosphate is normally formed during the glycolytic breakdown of glucose in the β-cell. Moreover, inhibition of glucose metabolism inhibits both Ca^{2+} uptake and insulin release.

Measurement of cytosolic free Ca^{2+}

Although there is good evidence that glucose stimulates insulin release secondary to a rise in cytosolic free Ca^{2+}, that evidence remained primarily based on indirect estimations. In our most recent experiments we have therefore attempted to measure cytosolic Ca^{2+} during stimulation of insulin release. The insulin producing cell is too small to be injected with the Ca^{2+} sensitive photoprotein aequorin, which has been used in larger cells such as muscle and nerve. The same consideration makes the use of Ca^{2+} selective, intracellular electrodes difficult. A third method first described for lymphocytes (13) proved useful. Quin 2 is a fluorescent Ca^{2+} indicator which increases its fluorescence upon Ca^{2+} binding. As the dissociation constant (K_D) for Ca^{2+} binding lies in the range of the cytosolic

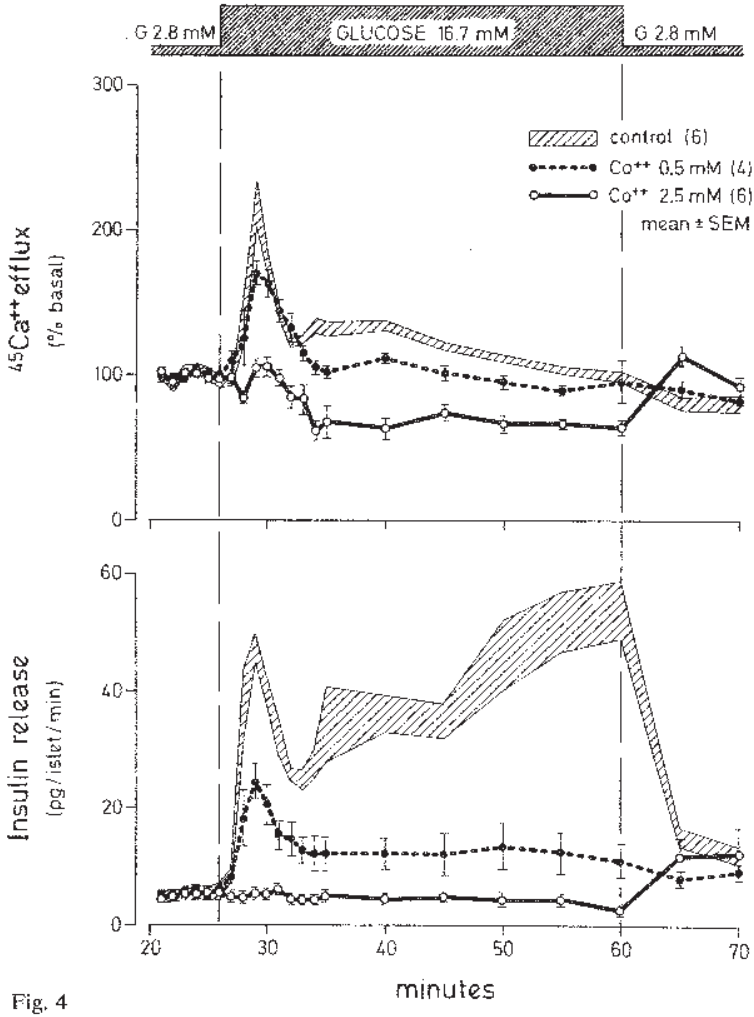


Fig. 4

Changes in glucose-induced insulin release and radioactive $^{45}Ca^{2+}$ efflux from islets in the presence and absence of cobalt ions (Co^{2+}). Co^{2+} blocks Ca^{2+} uptake by the islet cells. After 2 days preloading of isolated rat islets in tissue culture with $^{45}Ca^{2+}$, they were perifused for the measurement of $^{45}Ca^{2+}$ efflux (upper panel) and insulin release (lower panel). The shaded areas indicate the controls which were exposed to 16.7 mM glucose between minutes 26 and 60 of the perifusion. See reference 12.

Ca^{2+} concentration (10^{-7} M) quin 2 is a suitable indicator of cytosolic Ca^{2+}. As quin 2 itself is water-soluble and does not penetrate living cells, Tsien and his co-workers (13) described the use of a lipophilic ester of quin 2 which is taken up by cells. Once inside the cell the ester is hydrolysed by non-specific esterases and the water-soluble quin 2 remains trapped in the cell cytosol. The detection of the intracellular quin 2 fluorescence necessitates the recording of the fluorescence from a fairly large number of cells in suspension placed in a fluorometer cuvette. To this end, my colleague Dr. T. Pozzan, and I, used an insulin-producing cell line (RINm5F) derived from a rat insulinoma, since large amounts of cells in suspension can easily be obtained due to the high proliferative capacity of these cells. The same experimental approach is extremely difficult, if not impossible, in normal, non-tumoral, β-cells. However, there is at least one major disadvantage in the use of the cell line; it does not increase its rate of insulin release when exposed to high glucose concentrations. This may be due to an abnormal metabolism of glucose by the RINm5F cells mainly affecting the initial step in glucose metabolism, glucose phosphorylation (14). Fortunately, the triose glyceraldehyde, which enters the glucose breakdown pathway beyond the initial glucose phosphorylation step, is still able to elicit insulin release from these cells.

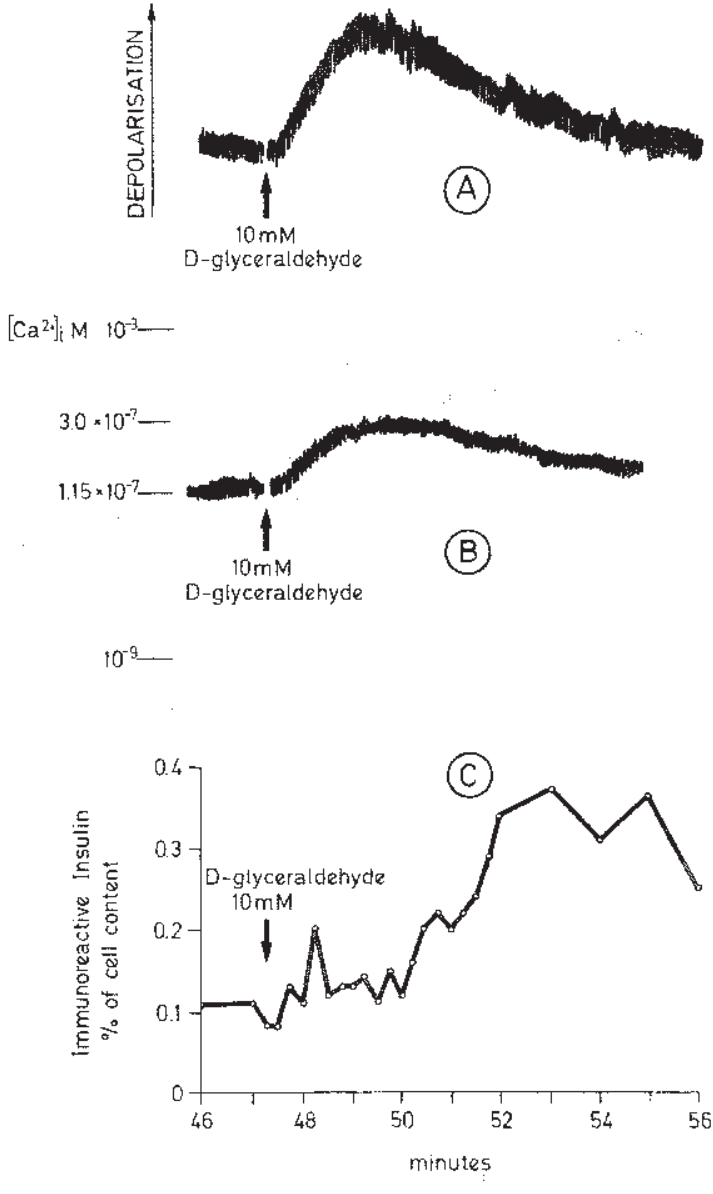
In the experiment shown in Fig. 5 glyceraldehyde-evoked changes in membrane potential, in cytosolic free Ca^{2+} and of insulin release were monitored in parallel on cells from the same original batch. Membrane potential was assessed with the fluorescent probe, bisoxonol, which increases its fluorescence as it is taken up by cells during depolarisation. Cytosolic Ca^{2+} was obtained by calibrating the quin 2 fluorescence. Glyceraldehyde first depolarized the cells and increased cytosolic free Ca^{2+} and then, after approximately one minute, the rate of insulin release increased. There is thus an elevation of the concentration of ionized Ca^{2+} in the cytosol of insulin producing cells which precedes the stimulation of insulin release. This situation is very similar to that found in muscle contraction, albeit on a different time scale (seconds rather than milliseconds), and fits well with the proposed role for Ca^{2+} as the coupling factor in stimulus-secretion coupling in the β-cell (15).

In skeletal muscle the source of calcium used to raise cytosolic Ca^{2+}

is an intracellular calcium storing organelle, the sarcoplasmic reticulum. In contrast, the more primitive muscle, the smooth muscle (e.g. uterus, intestine and vascular musculature), derives the Ca^{2+} necessary for contraction from the extracellular pool by opening of voltage-dependent or independent Ca^{2+} channels in the plasma membrane. In hormone secreting cells both intra- and extracellular calcium pools can apparently serve as a source. There is, for example, indirect evidence that glucose can mobilize Ca^{2+} from an intracellular compartment (7, 11). It has until now, however, not been possible to demonstrate such an effect of the glucose-like stimulus, glyceraldehyde, in our experiments with the insulin producing cell line and the Ca^{2+} indicator, quin 2. The reason for this may be that when the cells are incubated in the absence of extracellular Ca^{2+}, they rapidly lose Ca^{2+} from a labile, cellular calcium store which then is no longer available for mobilisation by the stimulus. I should like to illustrate Ca^{2+} mobilisation in another endocrine cell type.

In collaboration with Dr. W. Schlegel, we used the prolactin-secreting pituitary cell line, GH_3. Thyrotropin releasing hormone (TRH) binds to a plasma membrane receptor and stimulates the release of prolactin. The experiments shown in Fig. 6 which were performed in the absence of extracellular Ca^{2+}, demonstrate that TRH induces a rapid and transient increase in cytosolic free Ca^{2+}, as assessed with quin 2. Thus, even when the extracellular Ca^{2+} is lower than the cytosolic concentration of the ion, binding of a hormone to the cell surface can mobilize Ca^{2+} and lead to an increase of cytosolic Ca^{2+}. In contrast, when a high concentration of K^+ is added to the cells, the resulting depolarisation does not cause an increase of cytosolic Ca^{2+} in the absence of extracellular Ca^{2+} (Fig. 6). In the presence of

Fig. 5
Parallel changes in membrane potential (A), cytosolic free Ca^{2+} (B) and insulin release (C) induced by the triose glyceraldehyde. Cell suspensions of the insulin producing cell line (RINm5F) were preloaded with quin 2 acetoxymethylester (13) during 1 hour. Thereafter membrane potential changes were assessed with the fluorescent probe, bis-oxonol, and those in cytosolic Ca^{2+}, $[Ca^{2+}]_i$ with quin 2 fluorescence recorded in a fluorescence spectrophotometer. Insulin release from the cells in suspension was measured during perifusion. The arrows indicate the addition of glyceraldehyde, present continuously thereafter.


DEPOLARISATION
A
10mM
D-glyceraldehyde
[Ca2+]i M 10-3
3.0 ×10-7
1.15×10-7
B
10mM
D-glyceraldehyde
10-9
C
Immunoreactive Insulin
% of cell content
0.4
0.3
0.2
0.1
0
D-glyceraldehyde
10mM
46
48
50
52
54
56
minutes

physiological Ca^{2+} concentrations both TRH and K^{+} are able to raise the cytosolic Ca^{2+} level (not shown).

Intracellular calcium receptor proteins

How does the increase in cytosolic free Ca^{2+} induce the biological response? Again, muscle physiology has provided insights that have later been applied to other tissues. Troponin C, isolated from skeletal muscle, is part of the protein complex, troponin. Simplistically, in

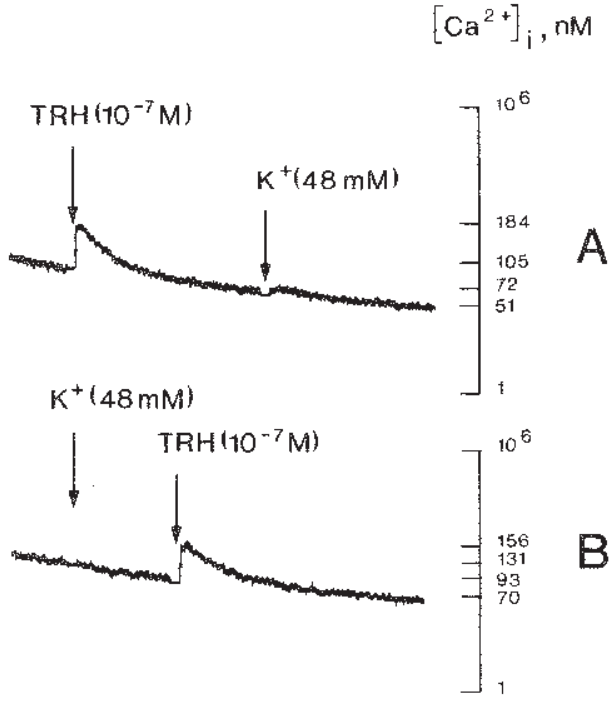


Fig. 6

Changes in cytosolic free Ca^{2+} induced by thyrotropin releasing hormone (TRH) in a prolactin producing cell line (GH_3). The cells were preloaded with quin 2 acetoxymethylester and then incubated in the absence of extracellular Ca^{2+}. Quin 2 fluorescence was recorded in a fluorescence spectrophotometer. 10^6 nM = 10^{-3}M Ca^{2+}.

the resting state, the contact between the contractile elements actin and heavy meromyosin, is blocked. When Ca^{2+} binds to troponin C, the constituents of the troponin complex are displaced and this, in turn, allows for the contact between actin and heavy meromyosin: the contraction takes place. Troponin C is thus a Ca^{2+}-binding regular protein (16). It belongs to a family of Ca^{2+}-binding regulator proteins with striking structural resemblance (homology of amino acid sequence). Another member of this family is calmodulin, first discovered by Cheung in 1970 as an activator of the enzyme which catalyzes the breakdown of cyclic AMP. It has now been identified in all nucleated cells (eucaryotic) and even in some bacteria. Its universal distribution at fairly high concentrations has been taken as an indication of its important regulatory role in cell function (17). Indeed, the list of enzymes activated by calmodulin and its involvement in various processes comprises as diverse functions as, e.g. smooth muscle contraction and fertilisation of the egg. Calmodulin has a molecular weight of about 17,000 daltons and can bind four Ca^{2+} ions. Upon Ca^{2+} binding the molecule changes its structure and can, in turn, bind to its target protein, usually an enzyme. Among the enzymes activated are protein kinases which transfer a phosphate group from adenosine triphosphate to a protein, for instance an enzyme which thereby may become active or inactive. Ca^{2+}-calmodulin is thus thought to induce smooth muscle contraction by the activation of myosin light chain kinase, the enzyme phosphorylating myosin light chains.

The presence of calmodulin in the β-cell has been demonstrated biochemically. It has been shown to activate at least five different enzymes in islet cell homogenates in a Ca^{2+} dependent manner (see Fig. 7) (18). Calmodulin not only transmits Ca^{2+} activation to cell components but is also involved in limiting the response, since it activates the Ca^{2+} ATPase in the plasma membrane, the enzyme which pumps Ca^{2+} out of the cell.

What evidence is there that calmodulin is involved in the stimulation of insulin release? The evidence remains circumstantial, since experiments directed to this question have to be carried out in intact cells and, to date, rely on the use of pharmacological inhibitors of calcium-calmodulin actions. One substance, the antipsychotic drug, trifluoperazine, inhibits both calmodulin activation of various islet cell

enzymes in the broken cell as well as glucose- or glyceraldehyde-stimulated insulin release from intact β-cells (19). In contrast, insulin release stimulated by agents which raise the concentration of the nucleotide cyclic AMP in the islet cells, e.g. the hormone glucagon or substances that inibit cyclic AMP breakdown, is not affected by trifluoperazine (18, 19). This finding suggests that calmodulin is not involved in the process of exocytosis of the insulin containing granules, provided that trifluoperazine penetrates to all areas where calmodulin is active.

Model for the secretion of insulin

A model for glucose-stimulated insulin release is shown in Fig. 7. In brief, glucose enters the β-cell freely and is then metabolized. The change of flux through a particular metabolic pathway (probably

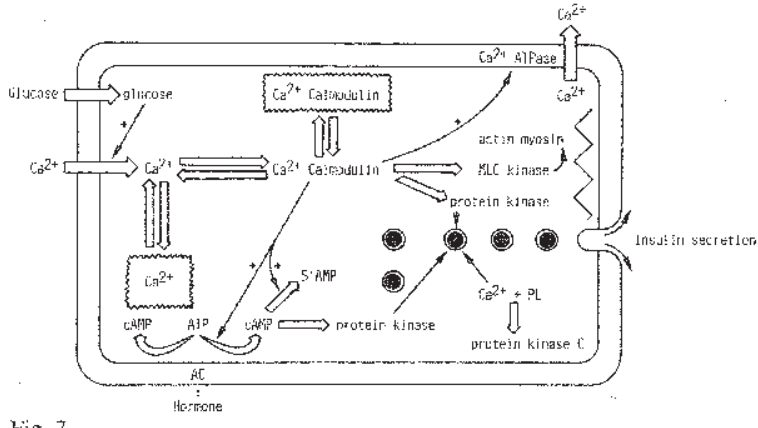


Fig. 7

Calmodulin directed functions in the β-cell and its possible involvement in insulin release. The Ca^{2+} regulator protein calmodulin is either bound to membranes or free in the cytosol. Calcium-calmodulin has been shown to activate adenylate cyclase (AC), cyclic nucleotide phosphodiesterase, Ca^{2+} ATPase, protein kinase and myosin light chain (MLC) kinase in islet homogenates (18). In addition, cyclic AMP dependent protein kinase and Ca^{2+} phospholipid (PL) activated protein kinase C, are present. The access of the insulin containing secretory granules to the plasma membrane is hampered by the actin filaments of the cell web (zig-zag line). Modified from S. Tomlinson et al. (Diabetologia *22*: 1, 1982).

glycolysis) generates a signal which decreases the potassium permeability of the β-cell membrane. This leads to depolarisation of the membrane (less potential difference between the inside and outside of the cell) and the opening of voltage-dependent Ca^{2+} channels. Ca^{2+} enters the cells passively down its electrochemical gradient and cytosolic free Ca^{2+} is increased (see also Fig. 5). Probably, but not yet demonstrated directly, there is also mobilisation of Ca^{2+} from calcium-storing intracellular organelles, such as endoplasmic reticulum. As the free Ca^{2+} concentration rises, Ca^{2+} binds to calmodulin and, perhaps, to other Ca^{2+} dependent regulatory proteins. This leads to the activation of several enzymes (depending on the degree of Ca^{2+} rise). Ca^{2+} dependent incorporation of phosphate into a key protein (not yet identified) catalyzed by protein kinase may then activate this key protein and prompt the insulin containing secretory granules to move towards the plasma membrane and, finally, to fuse the granule membrane with the plasma membrane leading to the extrusion of insulin into the extracellular space. These last steps may involve activation of contractile elements via myosin light chain kinase (interaction of actin with myosin) and/or a physical modification of the actin filament-containing cell web which normally acts as a submembranous barrier blocking the access of granules to the plasma membrane. In scavenger cells such as macrophages and white blood cells, a Ca^{2+} regulated protein, gelsolin, is involved in the breakdown of the actin network, thereby probably changing the motility of these cells (20).

Phosphorylation of the same putative key protein could also be brought about by two other means also indicated in Fig. 7: 1) the hormone stimulated generation of cyclic AMP and the subsequent activation of cyclic AMP dependent protein kinase; 2) substances like the neurotransmitter acetylcholine bind to receptors and cause breakdown of phospholipids, constituents of the plasma membrane. Some of these phospholipids are capable (in the presence of Ca^{2+}) of activating a specific protein phosphorylating enzyme called protein kinase C. The presence of this enzyme has been demonstrated recently in islet cell homogenates (21).

The aim of future research, then, is to answer the questions remaining unanswered in this model. The study of the physiopathology of insulin secretion is another obvious task. While it is known that con-

ditions with low Ca^{2+} levels in the blood, both in cows and man, are associated with impaired insulin release (7), very little is known about the most common form of defective insulin release encountered in patients with the maturity-onset type of diabetes mellitus (Type II). In this area intensive studies both on animal models of diabetes and clinical investigations are thus urgently needed.

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