all alternatives for the future of an urban system (this is possible if, through experience, we concentrate on the most important dimensions for every type of unit and every phase) and then eliminate the weakest ones. It is only in this way that we can avoid errors based on the mistaken belief that "I know," and can avoid the long period required for learning by trial and error, as primitive man learned.

This method certainly does not eliminate mistakes, but it reduces them to a minimum. Its application to the very difficult problem of the Urban Detroit Area (*11, 16*) has demonstrated how useful it can be for large-scale areas for which there is no human experience at all.

Experience has convinced me that, if we can develop a science of human settlements and, through it, recognize the guiding principles, laws, and procedures of man's action regarding terrestrial space, we can build much better human settlements in the future. This will be, not through the repetition of past solutions, but through their synthesis within the new frame formed on the basis of the new forces that have entered the game. The physical features of future cities can be at least as impressive as those of the famous cities of history or of today. At the same time, the guiding principle of real freedom of choice for everyone, not for certain classes only, can be implemented for the benefit of every person, and thus man's cities of the future can be better and far more important for all their inhabitants than the famous cities of the past.

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Cell Communication, Calcium Ion, and Cyclic Adenosine Monophosphate

Howard Rasmussen

Intercellular communication in higher animals takes place in one of two ways, either by direct communication from cell to cell by way of nerves or by indirect communication by way of chemical messengers within the circulatory systems. Traditionally, these two modes have been thought to differ. However, from the beginning of a serious consideration of the mechanisms underlying each, important similarities have been obvious. An immensely important similarity was discovered by Dale, Loeb, Gaddum, Elliott, Von Euler, and others (*1–5*): Transmission of signals from one cell to the next across synapses in the nervous system is generally by chemical rather than electrical means. It is recognized that synaptic transmission is a highly specialized function which is carried out by a very limited number of unique, low-molecular-weight neurotransmitters, whereas hormonal control in the endocrine system represents an amazingly complex and diversified set of functions carried out by a considerably greater number of different chemical messengers of varying size, structure, and chemical complexity. It had been assumed that the study of the action of a specific hormone and the release of neurotransmitter at a synaptic junction had little in common. However, recent experimental evidence indicates that neural transmission and the operation of many parts of the endocrine system, although differing in physiological function, may share a common biochemical mechanism at the cellular level. The basic elements in this widespread biochemical control mechanism are: calcium ions, adenosine 3′,5′-monophosphate (cyclic AMP), intracellular microtubules, microfilaments, secretory vesicles, and a class of enzymes known as protein kinases which phosphorylate specific proteins with adenosine triphosphate (ATP) as substrate.

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Intracellular Calcium

Ever since the early studies of Loeb, calcium ion has received the attention of cell biologists, physiologists, and biochemists because of the profound effects this ion has on cellular and enzyme function (*6, 7*). The most important and widely accepted role of calcium is that of serving as the coupling factor between excitation and contraction in all forms of muscle (*8, 9*), between excitation-secretion coupling at nerve endings (*2*), and in both exocrine and endocrine glands (*10–14*). Calcium ions are also involved in the regulation of glycogenolysis in muscle and ascites cells (*15–17*) and gluconeogenesis in kidney and possibly liver (*18–21*). It has been implicated as an intermediate in the action of melanocyte-stimulating hormone (*22*), in the action of vasopressin on the toad bladder (*23*), and in the action of parathyroid hormone on the function of bone cells (*20, 24, 25*).

In animal cells the concentration of calcium ion in the cytosol or free cytoplasm is in the range of 10^{-5} to

$10^{-8}M$ (*24–27*). In contrast, the concentration of this ion in mammalian extracellular fluid is close to $10^{-3}M$. Thus at all times the concentration ratio of calcium across the cell membrane (ratio of exterior Ca^{2+} concentration to interior Ca^{2+} concentration) is 10^2 to 10^5. The predicted ratio based on the Nernst equation and a membrane potential of − 80 millivolts (intracellular negative with respect to an extracellular reference electrode) is approximately 10^{-2}. This means that calcium ions are not distributed according to their electrochemical potential across the plasma membrane. Nevertheless, if cells are exposed to a solution of radioactive calcium (^{45}Ca), the radioisotope is readily taken up and lost by these cells—that is, calcium exchange takes place across this cell membrane. These facts have led to the conclusion that calcium enters the cell by a passive process, and is pumped out of the cell by an active process or calcium "pump." This notion has been considerably strengthend by the recent demonstration of a Ca^{2+}-activated adenosine triphosphatase in the plasma membrane of animal cells which is clearly distinct from the better-characterized $Na\text{-}K^+$-activated adenosine triphosphatase or sodium "pump" (*28, 29*).

Although the energy-dependent efflux of calcium is an important aspect of cellular calcium homeostasis, little is known about the biochemical regulation of this process. The presence of a distinct Ca^{2+}-activated adenosine triphosphatase has been definitely established only in the past few years (*28, 29*). It has been shown to have a low Michaelis constant (K_m) and high turnover for Ca^{2+}. The rate of passive calcium influx has been estimated to be from 0.01 to 0.10 picomole per square centimeter per second in a variety of different cells.

In addition to being transported across the membrane, calcium is a key component of the membrane (*30, 31*). Changes in calcium binding alter many of the physical properties of the membrane—for example, its permeability to water, other ions, and solutes, and its deformability (*26, 27, 30–35*). From a variety of evidence (*30–35*), a model of cell membrane activity has been constructed. The membrane is assumed to exist in one of two stable states, a resting or calcium-associated state and an active or calcium-dissociated state. An action potential is produced when the membrane shifts from the resting to the active state. This transition is brought about by dissociation of bound calcium which can be induced either electrically or chemically. During the active state the permeability of the membranes to Na^+, K^+, and Ca^{2+} is increased. The magnitude of the resulting changes in ion flux and intercellular ion concentrations depends on the rate and degree of calcium dissociation and on the concentration of the various ions in the intra- and extracellular fluid compartments.

In keeping with this model of cell membrane structure and function are recent observations concerning the electrical properties of cells which are stimulated chemically. Three basic electrical responses to chemical stimuli have been observed: (i) hyperpolarization, as seen following insulin action on muscle (*36*); (ii) depolarization, as seen after acetylcholine action on the chromaffin cells of the adrenal medulla (*10*), or hormone secretion from the adenohypophysis (*37*); or (iii) entrainment of a series of action potentials, a special kind of depolarization, as seen after glucose infusion into the beta cells of the islets of Langerhans (*38*).

Subcellular Calcium Exchange

Although the exchange of calcium across the cell membrane and its binding to this membrane are extremely important to cellular calcium homeostasis, they are not the sole means by which homeostasis is achieved. Two other cell organelles possess the ability to accumulate calcium, the mitochondria and microsomes. This accumulation is an energy-dependent process (*8, 39–43*). Isolated preparations of either are capable of reducing the calcium concentration in the media to $10^{-7}M$ or less, and it is assumed that these organelles have a similar capability in situ. In both cases, the bulk of the accumulated calcium is sequestered in a nonionized form. In spite of many similarities, there are significant differences between these two intracellular calcium "pumps." Nonetheless, both systems are capable of rapid calcium accumulation with rates as high as 200 to 2000 picomoles per square centimeter per second.

The physiological significance of the microsomal system in muscle cells is quite clear. In skeletal muscle, the sarcoplasmic reticulum is a highly developed and organized system which releases calcium upon excitation (*39*); the released calcium initiates contraction; the calcium pump in the sarcoplasmic reticulum then reaccumulates calcium, and thereby brings about relaxation (*8, 39*).

The physiological role of mitochondrial calcium accumulation is not yet well understood (*42*). It may be involved in (i) maintaining cytosol calcium at a low concentration; (ii) transcellular calcium transport; (iii) acting as a subsidiary system for regulating calcium exchange in contractile and secretory cells; or (iv) coordinating metabolic events in cytosol and mitochondrial matrix space.

Adenosine 3′,5′-Monophosphate

The first evidence of the existence and function of a cyclic nucleotide in animal cells was obtained nearly 14 years ago by Sutherland and Rall (*44*). They showed that cyclic AMP accumulated in liver as a result of epinephrine administration. Primarily from the work of Sutherland and his colleagues (*45*), the enzymatic mechanisms that are involved in both the synthesis and degradation of cyclic AMP are known.

Since its discovery, this cyclic nucleotide has been implicated as an intermediate in the action of many peptide hormones, in both exocrine and endocrine secretion (*45*), and in release of neurotransmitters at synapses and neuromuscular junctions (*46*). The enzyme responsible for cyclic AMP synthesis, adenyl cyclase, is bound to the plasma membrane of nearly all animal cells, whereas the enzyme responsible for its degradation, phosphodiesterase, is located in the cytosol, although in brain, part of the diesterase is clearly membrane bound. From this evidence, Sutherland and colleagues have developed the second messenger concept (*45*). In this concept, the first messenger, an external stimulus specific for the particular cell type, interacts with a membrane-bound adenyl cyclase leading to increased cyclic AMP synthesis within the cell. The increased concentration of cyclic AMP within the cell acts as a second messenger activating one or more processes or enzymes.

Among the amazing features of this control system are the remarkably diverse physiological consequences apparently invoked by an increase in second messenger within the cell. This complexity and diversity of final response, to a single second messenger, made it seem likely that the second messenger had a fundamentally different mechanism of action within each specific cell type. However, this no longer appears to be the case.

Protein Kinase and Cyclic AMP

Since the discovery of cyclic AMP, a matter of considerable interest has been its mode of action. The earliest, clearly identified effect of this nucleotide was on the activation of liver phosphorylase (*44*). It soon became evident that this effect was due to the phosphorylation of the enzyme phosphorylase. Largely due to the work of E. Krebs and his associates (*47*) the complete system underlying this conversion has been elucidated. The system consists of a series of enzymes, which exist in either an inactive or active form. Conversion from inactive to active form involves an energy-catalyzed phosphorylation. The first conversion involves phosphorylation of phosphorylase b kinase catalyzed by the enzyme phosphorylase b kinase kinase. This enzyme is activated by 10^{-9} to $10^{-7}M$ cyclic AMP.

When the basic aspects of this phosphorylase system and its relationship to cyclic AMP were elucidated, a search for its presence in other tissues was undertaken. It was found that the response of the adrenal cortex to adrenocorticotropic hormone involved cyclic AMP synthesis and phosphorylase activation (*48*). This led to the postulate that the major means by which 3′,5′-AMP regulated cellular metabolism was by controlling phosphorylase activity. However, this hypothesis was soon seriously challenged and proved to be inadequate to account for all the effects of cyclic AMP (*45*). There followed an increasing number of reports that 3′,5′-AMP was an allosteric activator of many different enzymes (*45*).

Although the phosphorylase system has been intensively investigated over the past 15 years, it has been considered a rather unique type of control mechanism. However, two discoveries have changed this view: (i) the discovery of many phosphoproteins, particularly in the cell nucleus, and the rapid turnover of their phosphate groups in vivo (*49–52*); and (ii) the demonstration that in nearly all tissues in which 3′,5′-AMP is a presumed second messenger following excitation, cyclic AMP–dependent protein kinases are present (*52–56*). Their presence has led quite naturally to the hypothesis that all the effects of cyclic AMP are mediated by controlling the activity of this class of enzymes.

Structural Basis of Secretion

Our knowledge of the structural basis of secretion has been greatly expanded by the use of the electron microscope. During the middle of the 1950's, application of improved fixation techniques for electron microscopy led to the discovery of microfilaments and microtubules in many different cell types, including nerve cells (*57–58*). The system of microfilaments and microtubules is particularly prominent in neurons and their axonal extensions. In the electron microscope, neurofilaments are approximately 100 angstroms in diameter and microtubules are 240 angstroms in diameter with a wall 50 angstroms thick. In longitudinal sections both structures appear to be straight, rigid, and unbranched. Neurofilaments have been shown to consist of two components, one similar to the subunits of microtubules and the other an acidic protein which may be responsible for the spikes or crossbridges observed in electron micrographs. Microtubules are composed of subunits with molecular weights of approximately 60,000 which have a high affinity for colchicine and which bind 1 mole of guanosine triphosphate per subunit. Depolymerization and repolymerization of microtubules can be observed in situ when cells are exposed to high hydrostatic pressure, low temperature, and colchicine. Although it was originally thought that microtubules and microfilaments were composed of similar proteins in different states of aggregation, this no longer appears to be the case. Microtubules do not bind heavy meromyosin, but microfilaments do bind this protein specifically (*59*). This specific binding of heavy meromyosin by the microfilaments is indicative of a marked similarity between these proteins and G-actin, the muscle protein.

It has been proposed that the secretion of endocrine or exocrine products involves the packaging of the material to be secreted into individual packets or vesicles (*2, 60–64*); that these vesicles accumulate at the site of secretion; and that excitation leads to a migration of vesicles to the cell surface where the membrane of the vesicle fuses with the plasma membrane of the cell with the concomitant secretion of the contents of the vesicle into the extracellular space. Although not universally accepted, there is increasing evidence that the vesicles are not free within the cell, but that their motion is restricted by the presence of a cytoskeleton primarily composed of microtubules (*65*). Furthermore, it has been proposed that the transport of the vesicles to the cell surface involves structural interactions with the microtubules. This notion is supported by the observation that colchicine, which binds specifically to microtubular protein (*66*), is able to block insulin secretion (*67*) and prevent vesicle migration in other systems (*65, 68, 69*).

Two possible roles of the microtubules in vesicle transport have been discussed: (i) microtubules form the structural element along which granule movement takes place but are not immediately involved in this mo-

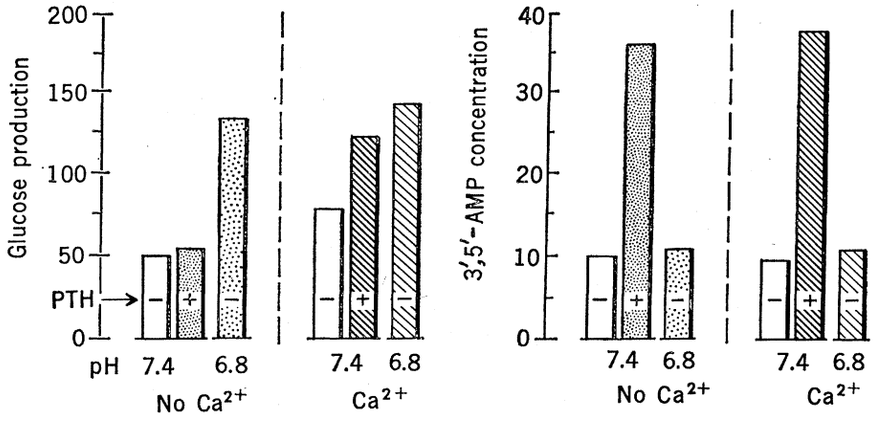

Fig. 1. The effect of parathyroid hormone (*PTH*) addition on 3′,5′-AMP concentration (expressed as the number of picomoles per milligram of protein) and glucose production (expressed as the number of nanomoles produced per milligram of protein per hour) in renal tubules in the presence and absence of 0.25 m*M* Ca^{2+} compared to the effect of increasing H^+ concentration from *p*H 7.4 to 6.8. The + and — indicate with and without parathyroid hormone, respectively.

Table 1. The role of Ca^{2+} and 3′,5′-AMP in cellular responses. Abbreviations are: GHRF, growth hormone releasing factor; LHRF, luteinizing hormone releasing factor; TRF, thyrotropin releasing factor; ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating factor; LH, luteinizing hormone; PTH, parathyroid hormone; and MSH, melanocyte stimulating hormone.

Cell	Stimulus	Response	Ca^{2+} required	3′,5′-AMP produced
Synapse	Electrical	Transmitter release	+	+
Neuromuscular junction	Electrical	Transmitter release	+	+
Anterior pituitary	GHRF	Growth hormone release	+	+
Anterior pituitary	LHRF	LH release	+	+
Anterior pituitary	TRF	TSH release	+	+
Posterior pituitary	Electrical	Vasopressin release	+	?
Salivary gland	Epinephrine	Amylase release	+	+
Beta cell, pancreas	Glucose	Insulin release	+	+
Adrenal cortex	ACTH	Steroid release	+	+
Adrenal medulla	Electrical	Epinephrine release	+	−
Exocrine pancreas	Acetylcholine	Amylase release	+	−
Liver	Glucagon	Glucose synthesis and release	?	+
Thyroid	TSH	Thyroxine release	+	+
Corpus luteum	LH	Progesterone release	+	+
Stomach	Histamine	HCl secretion	+	+
Heart	Epinephrine	Glycogenolysis	+	+
Toad bladder	Vasopressin	$Na^+ + H_2O$ transport	?	+
Kidney tubule	PTH	Gluconeogenesis	+	+
Melanocyte	MSH	Melanin dispersion	+	+
Slime mold	?	Aggregation	+	+
Sea urchin egg	Sperm	Fertilization	+	?
Adipocyte	Epinephrine	Lipolysis	+	+

tion; and (ii) they form the structural element and are intimately involved in the granule motion as well. In the former case, an additional component of the system is proposed—contractile filaments similar to or identical with microfilaments. In either case, the disruption of microtublar structure with colchicine would disrupt the structural framework of the system and lead to an inhibition of granule or vesicle migration.

Cyclic AMP and Cell Calcium

The widespread occurrence of both Ca^{2+} and 3′,5′-AMP as a coupling factor between cell excitation and response led to an investigation of their relationship (*70*). A relationship was observed in the salivary gland and renal tubule (*70*). Calcium ion and cyclic AMP are also involved in insulin and other endocrine secretion, peptide hormone action, and neuromuscular transmission (*19–21, 25, 70–77*). The number of systems in which both calcium and 3′,5′-AMP have been implicated as possible second messengers are listed in Table 1. In nearly all systems, excitation of the cell is followed by a rise in 3′,5′-AMP. In nearly all systems, this is accompanied by an increased uptake of calcium into the cell or an absolute requirement for Ca^{2+} in the external medium, or both, in order for the normal response to be observed.

Cyclic AMP production and external Ca^{2+} could be related in one of several ways (*70*). (i) Calcium is required for the specific stimulus to activate the adenyl cyclase; (ii) both calcium and 3′,5′-AMP have independent effects in the cell, and changes in their concentration occur simultaneously; or (iii) the intracellular action of 3′,5′-AMP requires the presence of Ca^{2+}. If the specific stimulus caused both an increase in 3′,5′-AMP synthesis and calcium ion uptake, these changes could either result from simultaneous but independent effects of the stimulus on cell membrane, or they could be sequential effects—for example, adenyl cyclase activation → 3′,5′-AMP production → increased calcium uptake.

In a considerable number of these systems, it has now been established

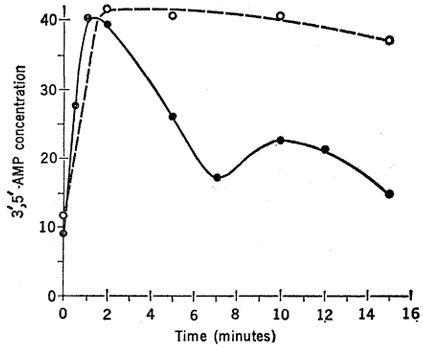

Fig. 2. The concentration of 3′,5′-AMP (expressed as the number of picomoles per milligram of protein) in isolated renal tubules as a function of time after addition of parathyroid hormone (zero time) in the presence (closed circles) and absence (open circles) of 2.5 m*M* Ca^{2+} [from Rasmussen and Nagata (*25*)].

that Ca^{2+} is not required for the specific stimulus to lead to an increase in 3′,5′-AMP. In these cases, the specific stimulus leads to 3′,5′-AMP production in either the presence or absence of Ca^{2+}, but the final physiological response is observed only in the presence of calcium. It is possible that a lack of external calcium so alters cell permeability that a disruption of intracellular systems or a leak of intracellular constituents into the medium, or both, takes place. However, this cannot be the explanation of events in the control of renal gluconeogenesis by Ca^{2+} and parathyroid hormone (*19–21, 25*). In this case the addition of parathyroid hormone leads to an increase in 3′,5′-AMP and a stimulation of glucose production from a variety of substrates. In the absence of Ca^{2+} in the medium, cyclic AMP production still takes place, but no increase in glucose formation is observed (Fig. 1). Renal gluconeogenesis is also stimulated by an increase in external H^+ (Fig. 1). In the absence of external Ca^{2+}, an increase in H^+ still causes a marked increase in glucose formation. Under these circumstances, a change in H^+ does not alter cyclic AMP concentration.

In isolated renal tubules, the addition of $10^{-5}M$ cyclic AMP produces a calcium-dependent stimulation of gluconeogenesis similar to that produced by parathyroid hormone. However, Borle (*78*) has shown that the hormone, but not the cyclic nucleotide, stimulates calcium uptake into renal cells. Several other cases have recently

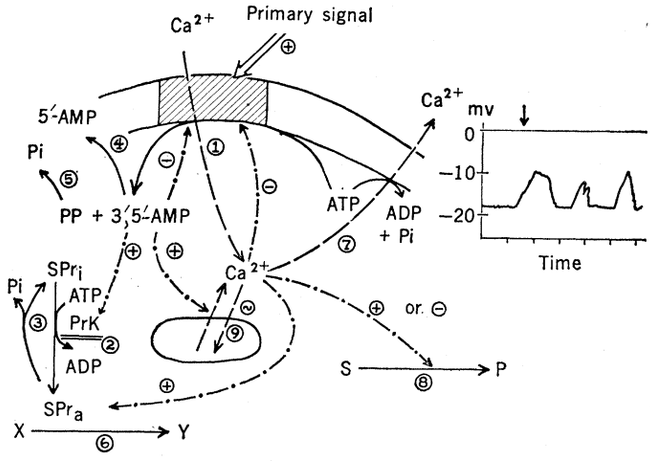

Fig. 3. The basic aspects of the adenyl cyclase control mechanism. The primary signal which can be either chemical or electrical leads to membrane depolarization or entrainment of a series of action potentials (inset) with the activation of the enzyme adenyl cyclase ①. This leads to an increase in 3′,5′-AMP and pyrophosphate (*PP*). The latter product is removed by the enzyme pyrophosphatase ⑤. The increase in 3′,5′-AMP leads to an activation of a protein kinase (*PrK*) ②, an enzyme that leads to the phosphorylation of one or more enzymatic or contractile proteins ⑥ within the specific cell. Two key enzymes that regulate the concentration of 3′,5′-AMP and phosphorylated protein are phosphodiesterase ④, which catalyzes the hydrolysis of 3′,5′-AMP to 5′-AMP, and protein phosphate phosphatase, which dephosphorylates the phosphorylated or active form of the protein or proteins ⑥ within the cell. The second control element in the system is Ca^{2+}. An increase in the concentration of Ca^{2+} in the cytoplasm is brought about by an increase in the uptake of calcium from the extracellular fluids or a mobilization of calcium from intracellular pools, or both. The mobilization of extracellular calcium is due to a direct effect of the stimulus on the plasma membrane of the cell, and the mobilization of intracellular calcium is brought about, with cyclic AMP acting on one or more intracellular membranes. The increased calcium within the cell serves one or possibly two functions. It is the specific activator of the phosphorylated protein (enzyme) ⑥ produced as a result of protein kinase ⑥ action. The increase in calcium may also activate other enzymatic reactions ⑧ within the cell, and it may act as a feedback inhibitor of further adenyl cyclase activation ①. The concentration of calcium within the cell is controlled not only by its leak into the cell, but by its active extrusion by a specific calcium "pump" or membrane-bound calcium-activated adenosine triphosphatase ⑦ and by its energy-dependent calcium accumulation in one or more cell organelles ⑨. The protein (SPr_a) phosphorylated by the protein kinase can be inactivated by a specific protein phosphorylase ③. The *X* and *Y* are substrate and product, respectively, of the reaction catalyzed by the active form of the enzyme SPr_a phosphorylated by the protein kinase; SPr_i, the inactive protein substrate for the protein kinase; *S* and *P* are substrate and product, respectively, of other calcium-activated enzymes. The + and − indicate positive and negative modification of the particular process, respectively.

been reported in which the specific peptide hormone which activates adenyl cyclase in a specific tissue also stimulates calcium uptake, but that addition of extracellular cyclic AMP does not stimulate calcium uptake even though it mimics hormone action. One of several conclusions is possible from this data. Either the effect of cyclic AMP does not require the presence of calcium and the cyclic nucleotide has no effect on calcium permeability; or the action of the cyclic nucleotide requires calcium, but the source of this calcium is an intracellular rather than extracellular pool. Much present evidence supports the latter point of view. In the isolated renal tubules, calcium exchange is rapid and incubation with [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA) leads to a depletion of intracellular calcium stores. Under these circumstances, cyclic AMP is ineffective in stimulating gluconeogenesis, but an increase in H^+ concentration will still enhance gluconeogenesis. Therefore, calcium is required for the action of this cyclic nucleotide in this and several other tissues, if the tissues are first depleted of intracellular calcium. This has been most recently demonstrated by Alm *et al.* (*79*) in the case of the response of isolated adipose tissue to epinephrine. The effectiveness of EGTA in depleting tissue stores of calcium varies considerably, depending on the rate of calcium fluxes across the cell membrane. These are rapid in a tissue such as kidney cortex, which is normally involved in calcium transport, and quite slow in a tissue like liver where calcium transport is not involved.

There is also direct evidence for an effect of cyclic AMP on the mobilization of calcium from intracellular pools, for an interaction of cyclic AMP with intracellular membranes, and for an effect of the cyclic nucleotide on the accumulation of calcium in isolated subcellular systems. One of the most important clues concerning the relation between intracellular calcium and cyclic AMP comes from studies with the xanthine drugs, theophylline and caffeine. These drugs alter calcium binding and flux in subcellular membranes (*80*) and inhibit the enzyme phosphodiesterase (*81*). The latter effect is thought to be due to their having a structural similarity to cyclic AMP. By analogy, it could be predicted that cyclic AMP causes the mobilization of calcium from intracellular pools. This has been demonstrated in toad bladder (*23*), liver (*82*), and slime mold. Also, the specific binding of 3′,5′-AMP to microsomal fraction from adrenal cortex and salivary gland has been described (*83*), and 3′,5′-AMP has also been shown to stimulate the phosphorylation of isolated microsomal membranes from kidney and brain (*84*). Finally, Entman *et al.* (*85*) have reported that cyclic AMP stimulates the uptake and rate of turnover of Ca^{2+} in the isolated sarcoplasmic reticulum from canine heart, and Schwartz and Walter (*23*) have shown effects of vasopressin and cyclic AMP on the calcium binding of particular fractions of toad bladder.

On the basis of this evidence, plus the fact that certain hormones stimulate calcium uptake into cells (see below) without activating adenyl cyclase, it now seems most likely that the interaction of the hormone with its receptor on a particular cell surface leads to the simultaneous increase in calcium permeability and adenyl cyclase activation. The subsequent increase in intracellular cyclic AMP acts as a positive feed forward activator within the cell by preventing the uptake into intracellular pools, or by mobilizing calcium from one or more intracellular pools, or both. This effect of cyclic AMP is undoubtedly of great importance in regulating the change in Ca^{2+} concentration in the cytoplasm, particularly in view of the marked difference in the flux of calcium across cellular and subcellular membrane discussed above.

A further, very interesting point concerning their relation is illustrated in Fig. 2. This shows the concentrations

of cyclic AMP in isolated renal tubules as a function of time after the addition of parathyroid hormone at zero time. When calcium was present in the external medium, the concentration rose rapidly to a value four times greater than that of the control, but then fell again and by 15 minutes was only 1.5 times the control value. In contrast, in the absence of calcium the concentration of cyclic AMP remained elevated throughout the entire 15 minutes. From several different lines of evidence, we concluded (*25*) that this difference is due to the fact that as the intracellular concentration of Ca^{2+} increases adenyl cyclase is inhibited. Thus, the hormonal response has its own feedback loop to prevent severe increases in intracellular calcium. If this is the correct interpretation, the data show that changes in the extracellular concentration of Ca^{2+} do not influence adenyl cyclase activity, but changes in intracellular or, more specifically, in cytoplasmic concentrations of Ca^{2+} do. From this it seems possible that the way in which hormones stimulate adenyl cyclase is by altering the content of an intramembranous pool of calcium in association with the enzyme, and with the pool of intracellular calcium in the cytoplasm. This is a particularly attractive possibility in view of the fact that Bradham *et al.* (*86*) have shown that Ca^{2+} has a dual effect on the adenyl cyclase of calf brain: stimulating at low concentrations in a Mg^{2+}-dependent reaction and inhibiting at higher concentrations.

The relation between 3′,5′-AMP and calcium is not confined to intercellular communication in higher organisms. A more primitive system of unusual interest is that of aggregation in the slime mold *Dictostylium discoideum*. This organism goes through a life cycle in which it exists initially as a motile single-cell amoeba. When unfavorable environmental conditions develop, hundreds of these individuals aggregate to form a slug which then differentiates into a spore. Through the work of Konijn, Bonner, and their collaborators (*87*), cyclic AMP has been established as the agent responsible for initiating aggregation. In our own laboratory (*88*), we have found that this effect of 3′,5′-AMP depends on Ca^{2+} in the external medium. Aggregation will not take place below a Ca^{2+} concentration of $10^{-6}M$ and is maximum when the Ca^{2+} concentration reaches $10^{-4}M$. A most important property of this system

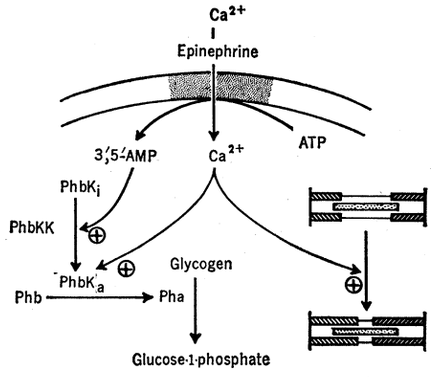

Fig. 4. The role of Ca^{2+} and 3′,5′-AMP in the epinephrine-induced increase in glycogenolysis and contraction (a positive inotropic effect) in cardiac muscle. Glucagon will also cause a similar activation of both glycogenolysis and has a positive inotropic effect. The positive inotropic response is due to an increased uptake of calcium by the cell, a direct result of hormonal stimulation, and a more efficient mobilization of the calcium stored in the sarcoplasmic reticulum due to the effect of 3′,5′-AMP. Abbreviations are: *PhbKK*, phosphorylase b kinase kinase; *Phb*, phosphorylase b; *Pha*, phosphorylase a; $PhbK_i$, inactive form of phosphorylase b kinase; and $PhbK_a$, active form of phosphorylase b kinase.

is that 3′,5′-AMP production is observed in low calcium ($10^{-6}M$) medium even though no aggregation is observed. In fact, in the absence of sufficient calcium, the production of 3′,5′-AMP is increased. This slime mold system is unique in that 3′,5′-AMP and Ca^{2+} are functioning as intercellular rather than intracellular messengers. This mechanism of primitive intercellular communication possesses the same basic properties as those of the intracellular mechanisms discussed above.

Dissociation of 3′,5′-AMP and Calcium

Many but not all systems in which 3′,5′-AMP appears to function also require external Ca^{2+}. An example of a case in which external Ca^{2+} is not necessary is the liver. In this organ, glucagon or epinephrine stimulate both gluconeogenesis and glycogenolysis by stimulating 3′,5′-AMP production (*89*). These effects are seen in the absence of external calcium. Nonetheless, Ca^{2+} may be involved in this case as well, but rather than being derived from an extracellular source it is mobilized from an intracellular source, an analogy to the variation in calcium source for excitation-contraction coupling in the different types of muscle. The evidence in favor of this viewpoint is of two kinds. Friedmann and Park (*82*) showed that 3′,5′-AMP or glucagon, when added to the fluid perfusing an isolated rat liver, led to a stimulation of calcium efflux from liver. In later work, Friedmann and Rasmussen (*90*) have found that this calcium efflux precedes the increase in glucose formation, and that it is blocked by prior treatment with tetracaine. Of particular importance, tetracaine treatment blocked the 3′,5′-AMP-induced increase in gluconeogenesis seen in livers from fasted rats and blocked the 3′,5′-AMP–induced increase in glycogenolysis seen in livers from fed animals, without greatly altering the basal rate of glucose synthesis. Tetracaine is one of a class of compounds, local anesthetics, which appear to act by altering calcium binding and calcium transport in cellular and subcellular membranes (*91*). Tetracaine inhibits excitation-contraction coupling and the metabolic effects of excitation in muscle. If it is acting similarly in liver, the results imply that the mobilization of calcium from an intracellular pool or pools is an integral aspect of the action of 3′,5′-AMP in stimulating glycogenolysis and gluconeogenesis in rat liver.

The Control System

In the light of the foregoing facts, a simple hypothesis can account for the mechanism of activation of many different cells by specific stimuli. This hypothesis is presented in Fig. 3. The basic elements of this system are two interrelated intracellular messengers, 3′,5′-AMP and Ca^{2+}. Activation or excitation of the cell leads to an increase in both. The increase in cyclic AMP activates a phosphorylating enzyme, or enzymes—that is, protein kinases and possibly phospholipid kinases. The products of the phosphorylation reactions catalyzed by these enzymes are now sensitive to Ca^{2+}. Their activation by Ca^{2+} leads either to enzymatic activation, to a change in their structure (for example, contraction), to a change in their interactions with other cellular constituents (for example, calcium bridges between phosphate groups on vesicle or membranes, or both), or to the determination of the membrane transport of calcium.

The response of the heart to epinephrine illustrates the way in which the

system functions in a specific case. Epinephrine stimulates 3′,5′-AMP production and calcium mobilization (*75*) in the heart. These lead, respectively, to sequential activation of phosphorylase b kinase kinase by phosphorylation of the inactive form, and phosphorylase kinase by direct activation (*92*) of the active form of the enzyme (Fig. 4). Both messengers are required to bring about an increase in glycogen breakdown; for example, in the absence of external Ca^{2+}, epinephrine still causes an increase in cyclic AMP production and an activation of phosphorylase b kinase, but no increase in phosphorylase a activity and glycogen breakdown.

An example of the operation of this control mechanism in endocrine secretion is that of insulin release from the beta cells of the pancreas. Glucose- or tolbutamide-induced insulin release requires the presence of calcium ion (*11*). Cyclic AMP is also involved in this process as shown by the work of Malaisse *et al.* (*93*), although the immediacy of its involvement has not yet been determined. In addition, Lacy (*67*) has shown that colchicine blocks glucose-induced insulin secretion and has proposed that microtubules are intimately involved in the secretory process. Of equal importance, Kuo and Greengard (*54*) have demonstrated a cyclic AMP–dependent protein kinase in pancreatic tissue, although they have not distinguished between exocrine and endocrine pancreas.

These observations can be incorporated into a model that accounts for insulin secretion (Fig. 5). Activation of adenyl cyclase is followed by the phosphorylation of a key component of the microtubular-vesicle system involved in secretion. This is followed by an activation of the system by Ca^{2+} which has been released into the cytosol of the cell. This model of secretion, if general, would explain the data of Yoshida *et al.* (*94*) in their study of amylase release from the isolated vesicles of salivary glands in which they demonstrated a need for both ATP and a protein factor from the cell supernatant in order for Ca^{2+} to increase amylase release, and the data of Selinger and Naim (*95*) showing that Ca^{2+} is required for both epinephrine and cyclic AMP–induced amylase secretion. However, on the basis of present evidence, it is not clear whether the cyclic AMP–dependent and Ca^{2+}-dependent events in secretion are necessarily always closely coupled in time.

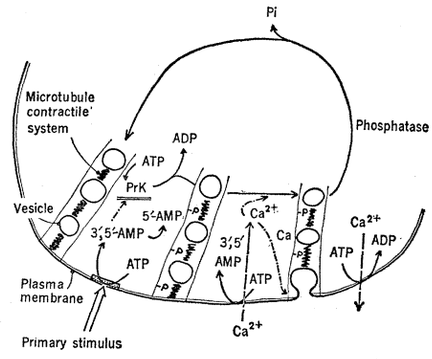

Fig. 5. A hypothetical model of the basic biochemical events in secretion. The stimulus of cell activation leads to an increased activity of adenyl cyclase with a consequent rise in 3′,5′-AMP. This intracellular messenger activates the enzyme protein kinase (*PrK*) leading to the phosphorylation of one or more elements in the cyto-skeleton-vesicle complex. This phosphorylation converts this complex from a calcium-insensitive to a calcium-sensitive state. The increased influx of calcium, also brought about directly or indirectly by the primary stimulus, now activates this complex and the vesicle moves to the cell surface. Contact between vesicle membrane and plasma membrane leads to fusion with subsequent discharge of vesicular contents. Calcium might play an additional role in this membrane-membrane interaction.

It seems most likely that during the evolutionary adaptation of this membrane control system to the regulation of specific cellular processes, a dissociation in time could occur between the effect of cyclic AMP and Ca^{2+}; for example, in the case of secretion the cyclic nucleotide-dependent step might be concerned with the movement of secretion granules from the center to the periphery of the cell, and Ca^{2+} in the final interaction of secretion granule and cell surface. This could be the case in insulin secretion, but the available evidence suggests that the role of cyclic AMP in amylase secretion is immediate.

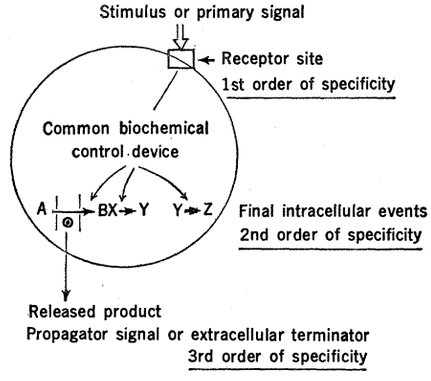

Fig. 6. The three orders of specificity which operate in conjunction with the common membrane control device and determine the unique cellular responses to specific stimuli. The *A*, *BX*, *Y*, and *Z* represent hypothetical metabolic intermediates.

The evidence for the operation of this control system in synaptic and neuromuscular transmission is considerable but as yet incomplete. It is well established that acetylcholine is stored in vesicles beneath the presynaptic membrane, and that its release requires the presence of external Ca^{2+} (*1*, *2*) and is inhibited by high concentrations of Mg^{2+}. Also, both adenyl cyclase and phosphodiesterase are present in very high concentrations in the nervous system, particularly in the synaptosome fraction of brain homogenates (*96*). Electrical stimulation of brain slices leads to an increase in their content of 3′,5′-AMP (*78*), and caffeine or theophylline, or both, augment transmitter release caused by electrical stimuli. Finally, a cyclic AMP–dependent protein kinase has been isolated in high yield from the brain (*54*) and has been shown to stimulate the phosphorylation of isolated microtubular protein from the brain (*97*). Finally, it has been shown that Ca^{2+} alone is not capable of provoking neurotransmitter release. After it was established that Ca^{2+} was required for transmitter release (*2*), Miledi and Slater (*98*) tried to demonstrate a direct role of Ca^{2+} in this process by the microinjection of calcium into the synaptic terminus. They were unable to elicit acetylcholine release. One of two conclusions is possible. Either Ca^{2+} is not a coupling factor in excitation-secretion coupling at the synapse; or it is a coupling factor but not the sole factor involved, and hence singly is incapable of activating the process.

Another feature of the generalized model (Fig. 3) is the aspect of time. The model as presently proposed is concerned primarily with the rapid and immediate responses of the respective tissues to specific stimuli. Yet in each of these cases, continued stimulation leads to prolonged activation. In many of these tissue responses, this would mean enhanced synthesis of protein, lipids, and in some cases even RNA. It is not clear whether these changes are brought about directly by Ca^{2+}, or 3′,5′-AMP, or both, or whether they are secondary to the primary changes induced by these messengers. However, in one instance, cyclic AMP–induced

phosphorylation of specific nuclear histone has been demonstrated in vivo (*54*). The relation of this phosphorylation to the changes in the physiologic function of the liver has not yet been delineated although it has been suggested that it is related to hormone-induced changes in enzyme concentrations. This problem has also been studied in relation to the stimulation of protein synthesis and amylase secretion in the parotid gland. Grand and Gross (*99*) have shown that epinephrine and dibutyryl 3′,5′-AMP stimulate both and present some data in support of the hypothesis that these two effects are both direct consequences of 3′,5′-AMP action. However, further work is necessary to establish this particular conclusion.

Apparent exceptions to the operation of this generalized control are the actions of acetylcholine on epinephrine secretion by the adrenal medulla and on amylase secretion by the pancreas (*10*). As shown in Table 1, calcium is required in both of these systems, but all evidence suggests that cyclic AMP is not a mediator of these responses. This casts doubt on the general importance of 3′,5′-AMP in the control of exocrine and endocrine secretion in other systems—for example, salivary gland secretion. However, a possible alternative is that a different cyclic nucleotide is involved in acetylcholine action. Another alternative is that, in the acetylcholine responsive systems, Ca^{2+} alone acts as the intracellular messenger.

Another apparent weakness of the present hypothesis is the frequent expression of doubt concerning the feasibility of 3′,5′-AMP's being able to function as a nearly universal second messenger in so many different tissue responses (*100*). Rephrased, the question would be an expression of concern about the ability of Ca^{2+} and 3′,5′-AMP to serve as nearly universal second messengers. These views overlook the several orders of specificity which can and do operate in unison with this single common biochemical control device (Fig. 6).

The first order of specificity resides at the cell surface where a highly specific selection of intercellular messenger operates. For example, glucagon will stimulate adenyl cyclase activity and promote hepatic gluconeogenesis, but not renal gluconeogenesis. Conversely, parathyroid hormone will act on the kidney, but not the liver.

An equally important second order of specificity is determined by the structural and biochemical uniqueness of each specific cell type. A simple example is the difference in response of liver and muscle to epinephrine action. This hormone activates glycogenolysis in both tissues, but the subsequent fate of the glucose-6-phosphate depends on the enzymatic profile of the two tissues. Because muscle lacks glucose-6-phosphatase, the major product of enhanced muscle glycogenolysis is lactic acid. In contrast, because glucose-6-phosphatase is present in liver, the major product is free glucose.

A third order of specificity also exists, and consists of three aspects—whether, where, and what. The first concerns whether or not the particular cell secretes a product in response to stimulation. The second concerns where the product is released—for example, into an exocrine duct, a synaptic cleft, or the blood stream. The third concerns what specific product is released from the particular cells. Employing these various orders of specificity, it is clear that the organism possesses potentially thousands of unique responses even though employing a common biochemical control device.

Conclusion

The hypothesis advanced in this article requires further validation. Undoubtedly it will require modification as our knowledge of biochemical control increases. Nevertheless, it should prove useful in focusing attention on the apparent similarity in the response of a large number of specific cell types to particular stimuli. Emphasis has been placed on a few common and apparently key elements in these responses. It is recognized that other factors are undoubtedly involved. Specifically, the changes in membrane potentials indicate the likelihood of widespread changes in the properties of the cell membrane, for example, changes in Na^+ and K^+ transport and distribution. These aspects of cellular responses may eventually prove to be of equal or greater importance than those common aspects of the system already identified.

The present hypothesis implies that a major part of the endocrine control systems involving peptide hormone secretion and peptide hormone action operate on the same basic cellular control system which regulates synaptic transmitter release as well as the actions of the neurotransmitters, epinephrine and norepinephrine, secreted by the sympathetic outflow of the autonomic nervous system. As such, this particular control system is clearly one of the most universal mechanisms for integrating the intracellular responses of animal cells to specific extracellular stimuli. A result of the elucidation of the general properties of this widespread cellular control mechanism is the realization that adaptation of cellular activities to new evolutionary stimuli has been achieved by subtle yet elegantly simple means. It is possible to recognize, in nature, beauty not only in form but in function.

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