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SYNARCHIC REGULATION AND SENSITIVITY MODULATION IN HORMONE ACTION*

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Summary

The present discussion has been concerned with an analysis of experimental data gathered largely in the past decade concerning the relationship of intracellular calcium ion to cAMP. From this analysis, the hypothesis developed is that Ca^{2+} and cAMP serve as interrelated second messengers when differentiated cells are called upon to perform their particular work function (contraction or secretion, etc.) by extracellular messengers (peptide or amine hormones, neurotransmitters) acting via specific cell surface receptors. To characterize and emphasize this duality the term synarchic regulation is introduced to convey the notion that these messengers act together to rule the intracellular domain. Although this duality appears to be nearly universal, the pattern of Ca^{2+}-cAMP interaction varies from cell type to cell type. At least five variations on the universal theme can be recognized: coordinate, hierarchical, redundant, antagonistic, and sequential.

By viewing hormone action as a process of information transfer, it is possible to analyze the sequential steps in hormone action. The key features of cell activation are the rise in concentration of a second messenger (Ca^{2+} or cAMP) in the cell cytosol, recognition of this rise by a specific receptor protein leading to a binding of messenger to protein, and then the binding of this complex in turn to one or more response elements. The molecular basis of this

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process in the calcium messenger system has been described and the very important relationship of change in cellular calcium metabolism to the messenger role of $(Ca^{2+})_c$ has been emphasized. A distinction has been made between this process of amplitude modulation of cell function in the calcium messenger system, and the process of sensitivity modulation. This occurs by a mechanism that involves the activation of a calcium-calmodulin-dependent enzyme by changing its sensitivity to activation by Ca^{2+} rather than a change in $(Ca^{2+})_c$. A common means by which sensitivity modulation takes place in the calcium messenger system is by a cAMP-dependent phosphorylation of a subunit of a calmodulin modulated response element. This type of sensitivity modulation provides molecular evidence in support of the concept of synarchic regulation.

Introduction

For the generation of endocrinologists educated in the past two decades, the elegant simplicity and satisfying universality of the second messenger concept of peptide and amine hormone action has provided the major intellectual framework within which actions of this group of extracellular messengers have been considered (1). The second messenger model has found its way into nearly all textbooks of physiology, biology and endocrinology. In one sense, this was inevitable, in another unfortunate. Inevitable because it reduced a very complex and diverse set of cause and effect relationships to a simple easily understood model. Unfortunate, because it led to the belief that all such relationships had been explained, and further critical analysis of them would not lead to significant new insights into the general problem of how this group of agents regulate cell function.

It is, then, with some trepidation that I plan to present to you as the major theme of this lecture a challenge to this accepted dogma, and to propose an alternative model of how this class of agents regulates cell function.

Before doing so, it is necessary to remind you of the fact that standing in contrast to the second messenger model of hormone action is the model of calcium ion as coupling factor in stimulus-response coupling in nerve, muscle, and exocrine gland (2, 3). These two separate views of how endocrine (non-excitable) and neuro-muscular (excitable) cells are activated can be presented schematically as in Figure 1. I shall try to convince you that this separation is totally artificial; and that Ca^{2+} has a major messenger function in non-excitable cells, and cAMP in excitable cells (4-6). Rather than separate systems controlling response in physiologically distinct kinds of cells, the available data indicate that there is a single universal system for coupling stimulus to response (5). This single system employs both Ca^{2+} and cAMP

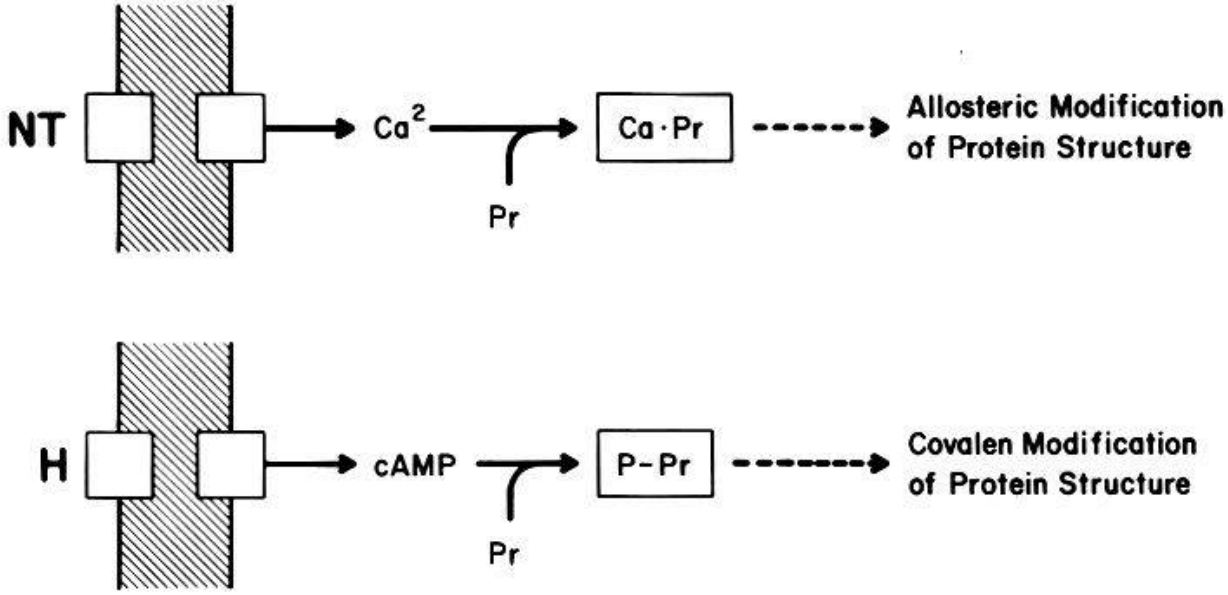

Fig. 1. The classic models of neurotransmitter (NT) and peptide hormone (H) action. Neurotransmitters act to increase intracellular calcium concentration which binds to a calcium receptor protein to induce allosteric modifications in other proteins. Hormones act to increase cAMP concentration which binds to the regulatory subunit of a protein kinase causing its activation and leading to covalent modifications of the structure of other proteins.

as intracellular messengers. Synarchic regulation has been coined as a term to describe this relationship, and to emphasize that Ca^{2+} and cAMP act together (syn) as rulers or heralds (archons) of the intracellular domain (5).

Synarchic Regulation

From an historical point of view, three observations made approximately a decade ago where clues to the recognition of the intimate relationship between Ca^{2+} and cAMP. One was the discovery by Namm and Meyer that the effect of epinephrine on cardiac glycogenolysis was Ca^{2+} dependent, another was the observation by Nagata and myself (8) that both Ca^{2+} and cAMP played a messenger role in the action of parathyroid hormone on renal gluconeogenesis, and the third the discoveries of Cheung (9) and of Kakiuchi (10) that the hydrolysis of cAMP by brain phosphodiesterase was regulated by Ca^{2+}. From these discoveries and a consideration of much other data showing that Ca^{2+} and cAMP appeared to function in a number of the same tissue, I proposed in 1970 that they were components of a common cellular control device (11). The data obtained in the last decade has largely substantiated this hypothesis, but has also led to a much better appreciation of both the universal and parochial aspects of its function (5). It is much more elaborate, elegant, and adaptable than I had envisioned.

Of crucial importance to the development of this hypothesis were the experiments that Berridge, Prince and I conducted on the fly salivary gland (12, 13). These experiments established that in regulating fluid secretion in this gland, serotonin (5HT) employed both Ca^{2+} and cAMP as intracellular messengers. The subsequent work of Berridge and his colleagues (6, 14) has reenforced the basic correctness of our original model. This model is that when 5HT acts on the fly salivary gland there is an increase in the concentration of both Ca^{2+} and cAMP within the cell, and these two messengers acting upon the Cl^- channel and the K^+ pump, respectively, in the luminal cell membrane stimulate the transcellular movement of KCl and consequently that of H_2o, i.e. fluid and electrolyte secretion. Their (the intracellular messengers) pattern of interaction can be said to exemplify (coordinate control) of cellular response (Fig. 2).

For a time, we considered the possibility that this might be a universal model. However, it soon became apparent that in other tissues although both cAMP and Ca^{2+} played a role in coupling stimuli to response, their interrelationships were dissimilar from that seen in the fly salivary gland. Control of response in four different tissues will be discussed as examples of other patters of Ca^{2+}-cAMP interactions. (See Rasmussen (5) and Rasmussen and Waisman (15) for a complete citation of literature).

The first of these is the hormonal control of glucose production by the liver. This is the system in which Sutherland and Rall first elucidated the second messenger role of cAMP in the actions of glucagon and epinephrine (1). Work since their initial studies has shown that α-adrenergic agonists, vasopressin, and angiotensin also stimulate glucose production, but by a calcium-dependent, cAMP-independent mechanism. There is then two different groups of hormone acting via two apparently different intracellular messenger systems. When they do so, they regulate the same enzymatic steps in the gluconeogenic and glycogenolytic pathways. A reasonable working hypothesis is one in which control of hepatic glucose production is modulated by the Ca^{2+} and cAMP messenger systems acting in a redundant fashion (Fig. 2). Either messenger acting via specific and different protein kinases catalyzes the phosphorylation of the same key regulatory enzymes in these metabolic sequences.

A similar type of redundant pattern of control is seen in the actions of ACTH and angiotensin II on the adrenal glomerulosa: ACTH acting via the cAMP and angiotensin via the Ca^{2+} messenger system control aldosterone biosynthesis and secretion.

To show that specific pattems of cAMP and Ca^{2+} are seen in neuro-muscular systems as well as endocrine ones, and at the same time illustrate a hierarchical pattern of synarchic regulation (Fig. 2), one can consider the neural control of contraction of the accessory radula closer (ARC) muscle in the Aplysia (16). This muscle receives a dual innervation. Cholinergic

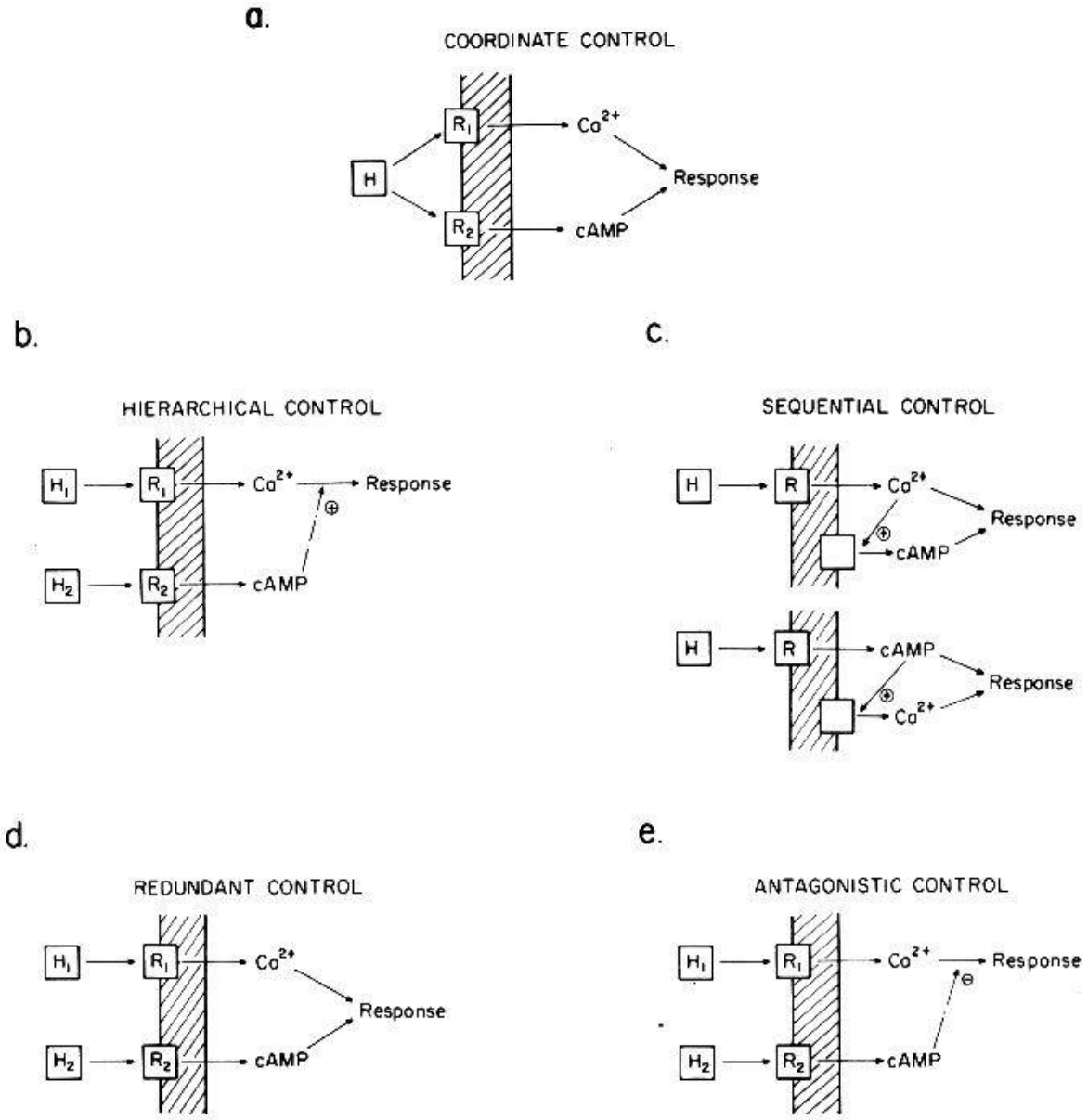

Fig. 2. The various recognized patterns of synarchic regulation. See text for discussion.

neurons, acting via the Ca^{2+} messenger system, induce contraction. This is operationally similar to what is seen in various forms of mammalian muscle. Serotonergic neurons when stimulated in a non-contracting ARC have no apparent effect on either membrane potential or contraction. However, if these neurons are stimulated just prior to the stimulation of the cholinergic neurons, the subsequent contraction is considerably enhanced. The biochemical consequence of serotonergic neuron stimulation is an activation of adenylate cyclase leading to a rise in intracellular cAMP. Exogenous cAMP mimics the effect of serotonergic neuron stimulation. Thus, stimulation of these neurons lead to the generation of a cAMP message which enhances the effectiveness of the Ca^{2+} messenger arising as a consequence of

cholinergic neuron action. These two inputs acting via their separate intracellular messengers exhibit a hierarchical pattern in determining cell response.

Similar hierarchical patterns are seen in many endocrine systems. One of the most thoroughly studied is the control of insulin secretion (17, 18). In this system, changes in extra-cellular glucose concentration control the calcium messenger system, and act as the primary modulator of insulin secretion. However, agents like glucagon which act to enhance the cAMP content of the insulin secreting cells, and α-adrenergic agonists which act to decrease the cAMP content, determine the effectiveness of a given change in glucose concentration in altering insulin secretion. A rise in (cAMP) leads to an enhancement of the action of glucose, a fall to a diminution of the action of glucose. This behavior of this dual metabolic control of insulin secretion is operationally similar to the dual neural control of ARC contraction. This type of hierarchical interaction between the Ca^{2+} and cAMP messenger systems is one of the most common of the different patterns under discussion (5).

In contrast to the situation in the ARC muscle where a rise in cAMP brings about an enhancement of the effect of the calcium messenger system, in mammalian smooth muscle a rise in cAMP acts in opposition to a rise in Ca^{2+} (19-21). In this type of antagonistic pattern (Fig. 2), stimulation of a cholinergic neuron acting via the Ca^{2+} messenger system induces a contractile response, and stimulation of β-adrenergic neurons acting via the cAMP messenger system induces relaxation. The cellular basis of cAMP action consists both of decreasing the (Ca^{2+}) in the muscle cell, and of changing the sensitivity of the Ca^{2+}-response element to activation by Ca^{2+}. The molecular basis of this latter effect will be discussed below. At this point, the important concept is that in smooth muscle Ca^{2+} and cAMP are interacting messengers as in the other systems discussed above, but their pattern of interaction in this particular case is an antagonistic one.

The final example is that of the control of catecholamine biosynthesis and secretion from the adrenal medulla (22). Secretion is provoked by cholinergic stimulation, and Ca^{2+} is the intracellular messenger which couples stimulation to the immediate secretory response (3). If the gland is subjected to sustained stimulation, there is an increase in cAMP. This rise in (cAMP) is transient, but nonetheless of critical importance in inducing the synthesis (via nuclear gene activation) of tyrosine hydroxylase a key enzyme in the pathway of catecholamine biosynthesis. This rise in (cAMP), rather than occurring as a consequence of direct neurotransmitter activation of adenylate cyclase, is apparently due to a calcium-calmodulin activation of the cyclase. Likewise, calcium-calmodulin apparently mediates the subsequent fall in (cAMP) by activating the enzyme, phosphodiesterase, responsible for cAMP hydrolysis. These data imply that in the activation of catecholamine secretion and biosynthesis both Ca^{2+} and

cAMP serve as intracellular messengers, and that their pattern of interaction is a sequential one (Fig. 2). An initial rise in (Ca^{2+}) induced by neurotransmitter action induces the rise in (cAMP).

The various recognized patterns of synarchic regulation are depicted schematically in Figure 2. The point to be emphasized is that in each cell type representing each pattern type, Ca^{2+} and cAMP serve as interrelated and interacting second messengers, but that within the framework of this universal theme there are a number of particular variations. Equally important has been the new insights into the molecular basis of cell regulation that have come from this new frame of reference. To consider these insights it is first necessary to consider hormone and neurotransmitter action in terms of information transfer systems (15).

Modulation of Cell Function

Considered as an information transfer process, hormone action can be broken down into at least seven sequential steps: 1) recognition of extracellular messenger (hormone) by a specific surface receptor; 2) transduction of extracellular into intracellular messenger; 3) transmission of intracellular messenger from cell surface to cell interior; 4) reception of intracellular messenger by a specific receptor protein; 5) modulation of the activity of one or more response elements by the messenger-receptor complex; 6) response of these elements; and 7) termination of messenger and/or response.

Of particular interest to the present discussion are the steps of reception, modulation and response. These features of the Ca^{2+} messenger system will be considered in some detail because they are fairly well understood at the molecular and cellular levels, and this understanding has provided an important confirmation of the importance of the Ca^{2+}-cAMP relationships.

In the classic second messenger model of hormone action, or in the classic model of calcium as the factor coupling stimulus to response, the key event is a rise in the concentration (amplitude) of the intracellular messenger (message). This change in amplitude is detected by an appropriate receptor protein which in turn controls the function of other proteins, response elements. This type of model can be considered to exemplify the process of amplitude modulation (Fig. 3). However, as presented it is much too simplistic. Recent studies of the kinetic properties of calmodulin regulated reactions (23-27), as well as those of cellular calcium metabolism and homeostasis (28-30), provide the information from which a more complete model of this process can be constructed.

The critical feature to be explained by this model is what one might call the calcium paradox.

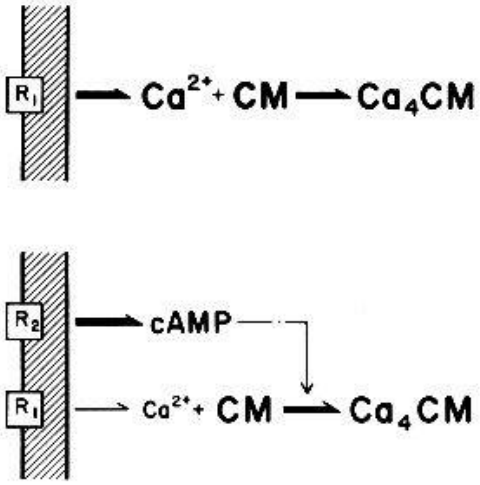

Fig. 3. The contrast between amplitude (upper) and sensitivity modulation in the calcium messenger system. See text for discussion.

This paradox arises from the following two sets of facts: on the one hand, the cell has developed an extremely elaborate set of mechanisms for maintaining cellular calcium homeostasis and for keeping the cytosolic calcium ion concentration $(Ca^{2+})_c$ constant (28-30) and on the other, it employs changes in the $(Ca^{2+})_c$ as a major means of communicating information from cell surface to cell interior (5). The paradox is how it achieves the latter in the face of the former. A corollary to this paradox is that one very important reason for controlling cellular calcium exchange so precisely is the constant threat of calcium intoxication. Excess cellular calcium leads to cell dysfunction and cell death (31-33).

The problem faced by the cell is that the extracellular $(Ca^{2+})_c$ is 100 μM, and the calcium ion concentration $(Ca^{2+})_c$ in the cytosol of the resting cell is 0.2 μM (28). There is a 5000-fold concentration gradient of Ca^{2+} across the plasma membrane (Fig. 4). This gradient is maintained by three processes in this membrane: 1) a very low permeability to Ca^{2+} so influx is limited (circa 4 μmoles/kg cell H_2O/min); 2) a Na^+-Ca^{2+} exchange system in which extracellular Na^+ exchange for intracellular Ca^{2+}--this is a process of secondary active transport in which the energy in the Na^+ gradient is used to drive Ca^{2+} out of the cell against its concentration gradient; and 3) a specific ATP-dependent calcium pump (Ca^{2+}-Mg^{2+} ATPase) by which the hydrolysis of ATP is used to drive the concentrative efflux of Ca^{2+} from the cell.

Two other intracellular processes also serve to keep $(Ca^{2+})_c$ constant. The first is a poorly defined cytosolic buffer system (Fig. 5). Even though $(Ca^{2+})_c$ is in the range of 0.2-2.0 μM, the total calcium in the cytosol is 50-150 μM (28). Even if we employ the lower figure, this means that greater than 95 percent of the cytosolic calcium is bound. Any sudden change in $(Ca^{2+})_c$ is buffered by this system. In addition, the mitochondria possess an enormous capacity

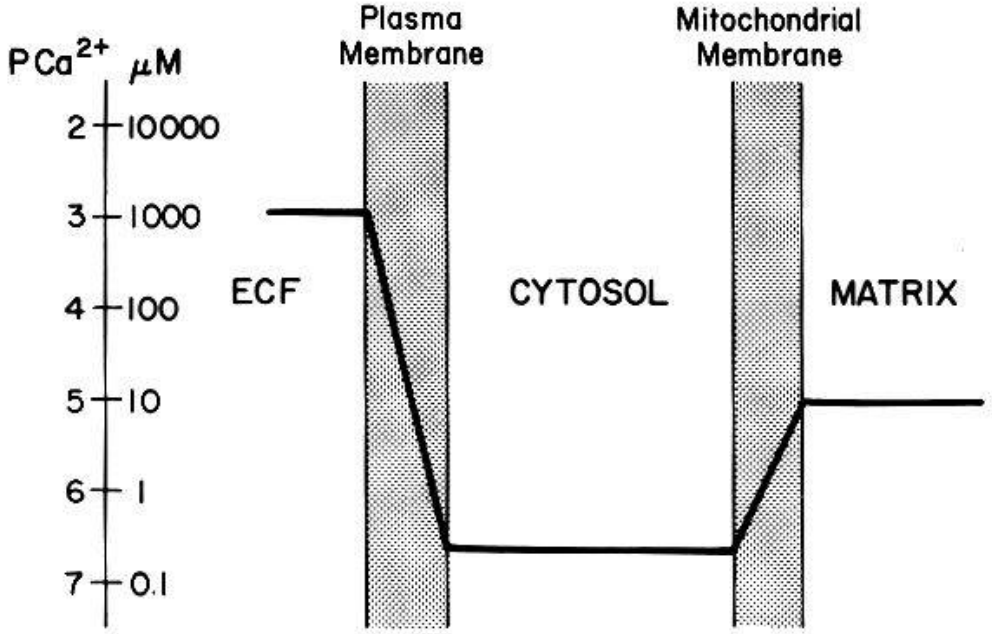

Fig. 4. The calcium ion concentration profiles across the plasma and mitochondrial membranes of the cell. The calcium ion concentration in the mitochondrial matrix space is an estimated value.

to accumulate calcium (28). There are both an active uptake or influx pathway and a separate efflux pathway of Ca^{2+} transport across the mitochondrial membrane (34). These function so that at a $(Ca^{2+})_c$ of 0.8 μM, in isolated mitochondria (34), or closer to 0.2 μM in mitochondria in situ (35), efflux and influx are balanced. As soon as $(Ca^{2+})_c$ increases to 0.4 μM or greater there is a net active uptake of Ca^{2+} by the mitochondria. The Ca^{2+}-activation curve of the mitochondrial Ca^{2+} pumps is rather steep so as $(Ca^{2+})_c$ rises the rate of net accumulation of calcium by these organelles increases. This mitochondrial system serves as the main calcium sink protecting the cell from calcium intoxication because most of the accumulated calcium is deposited in a non-ionic form (a complex of calcium, phosphate and ATP) (Fig. 5). However, this mitochondrial sink has a limited storage capacity (Fig. 5). In the resting cell the exchangeable calcium pool in the mitochondria is about 150 μmoles/kg cell H_2O (28) and the total capacity is about 6000 μmoles/kg cell (31).

The constraints placed on using Ca^{2+} as an intracellular messenger are defined in part by this behavior of the mitochondria. For example, if during activation of a cell by a hormone, the $(Ca^{2+})_c$ rose to 10 μM, and stayed there, and the mitochondria function as predicted from their known properties, their calcium accumulating capacity would be exceeded in a little over 1.5 hrs (Table 1). If $(Ca^{2+})_c$ rose to 1.0 μM and remained there during sustained cellular activation, the mitochondrial sink would fill in approximately 20 hrs.

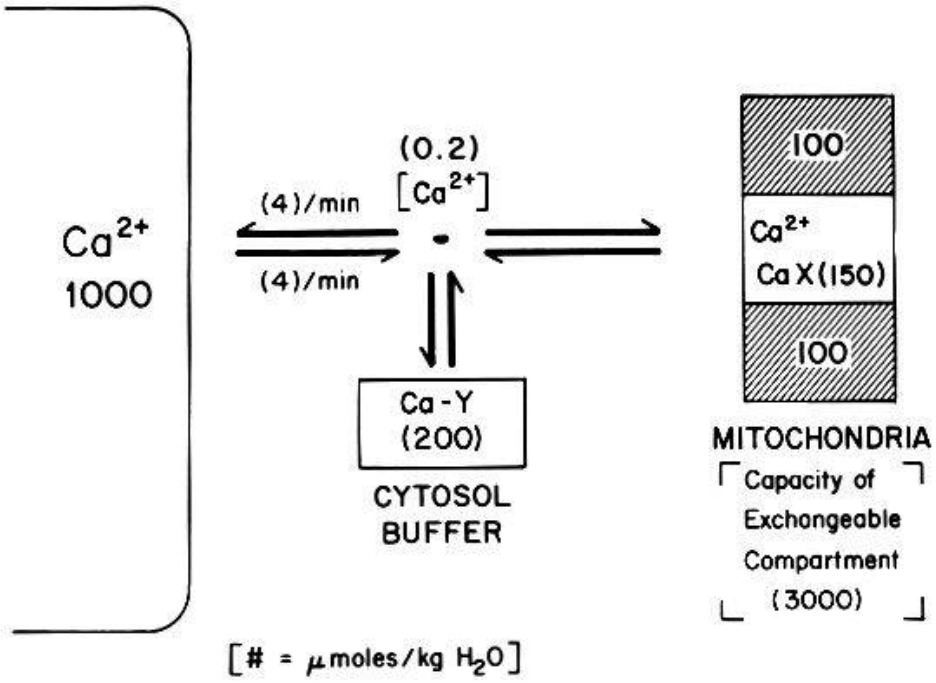

Fig. 5. The size of various calcium pools within the cell. See text for discussion.

	FLUX RATE				
$[Ca^{2+}]_c$	(R) 0.2 μM	10 μM	1.0 μM	0.4 μM	(A) 0.3μM
	μmoles/kg cell H_2O/minute				
CELL					
Influx	4	100	30	20.5	20.0
Efflux	4	40	24	18.5	18.2
Net	0	60	6.0	1.5	1.8
MITOCHONDRIA					
Influx	0.5	40.5	5.5	1.8	2.0
Efflux	0.5	0.5	0.5	0.5	0.5
Net	0	40.0	5.0	1.3	1.5
CELL DEATH (hrs)	–	1.5	20	75	55

Table 1. Estimated flux rates of calcium into and out of the cell, and into and out of mitochondria under various cellular conditions. Column 1--a resting (R) cell with a cytosolic calcium ion concentration $(Ca^{2+})_c$ of 0.2 μM; column 2--a cell with a $(Ca^{2+})_c$ of 10 μM; column 3--a cell with a $(Ca^{2+})_c$ of 1.0 μM; column 4--a cell with a $(Ca^{2+})_c$ of 0.4 μM; and column 5--an activated (A) cell with a $(CA^{2+})_c$ of 0.3 μM. At the bottom are given estimates of the time till cell death under each of these conditions.

These calculations are imprecise because they are based on several assumptions, but they do illustrate the nature of the problem. If a cell is to employ Ca^{2+} as an intracellular messenger, it must do so in such a way as to avoid calcium intoxication. That this is a real rather than imagined problem is best illustrated by the observations of Fleckenstein. He showed that rats treated for a number of hours with high doses of isoproterenol developed severe cardiac dysfunction and died because of the accumulation of excess calcium by the myocardial cells, and in particular their mitochondria (32).

With these considerations as background, it is now possible to discuss a model of amplitude modulation in the calcium messenger system. An idealized cell exhibiting a sustained cellular response to the sustained application of a hormone will be considered. When the hormone interacts with its surface receptor, it causes a release of Ca^{2+} bound to the plasma membrane, and an increased entry of calcium into the cell. As a consequence, the $(Ca^{2+})_c$ in the cytosol rises, the Ca^{2+} binds to calmodulin (CM) and the response is initiated. There are several unexpected features seen in this activated cell. First, even though the response is sustained, the rise in $(Ca^{2+})_c$ is transient (29). The $(Ca^{2+})_c$ rises from 0.2 to 1.0 μM then after a few minutes it falls to a value of 0,3 - 0.4 μM. Second, even though the rise in $(Ca^{2+})_c$ is transitory, the increase in Ca^{2+} influx into the cell is sustained (28). This increased rate of influx is coupled to an increased rate of efflux of Ca^{2+} back out of the cell indicating that the Ca^{2+} pump in the plasma membrane has been activated (36-38). Even so, influx exceeds efflux so there is a net accumulation of Ca^{2+} by the cell, and particularly the mitochondria. In 6 hrs this leads to a 2.5-fold increase in cellular calcium (30).

The sustained response to the transitory rise in $(Ca^{2+})_c$ is explained by the molecular properties of calmodulin regulated enzymes (23-27). The interaction of calcium, calmodulin, and response elements can be considered as an ordered, highly cooperative sequence of reactions.

(1) $Ca^{2+} \cdot CM \rightleftarrows Ca \cdot CM$

(2) $Ca^{2+} + Ca \cdot CM \rightleftarrows Ca_2 \cdot CM$

(3) $RE + Ca_2 \cdot CM \rightleftarrows Ca_2 \cdot CM \cdot RE$

(4) $Ca^{2+} + Ca_2 \cdot CM \cdot RE \rightleftarrows Ca_3 \cdot CM \cdot RE$

(5) $Ca^{2+} + Ca_3 \cdot CM \cdot RE \rightleftarrows Ca_4 \cdot CM \cdot RE^*$

where $Ca_4 \cdot CM \cdot RE^*$ is the activated response element (RE).

These five reactions can be depicted in a three step model (Fig. 6). The first step consists of the cooperative binding of the first two calcium to calmodulin. Cooperative binding means that once the first Ca^{2+} is bound, the second binds with a higher affinity. The second step is the binding of a response element (RE) to $Ca_2 \cdot CM$, the form of calmodulin having two of its four binding sites filled. The binding of the first two calciums to CM greatly enhances the

THE ULTIMATE CONVERSATION IN HORMONE ACTION VIA SYNARCHIC REGULATION

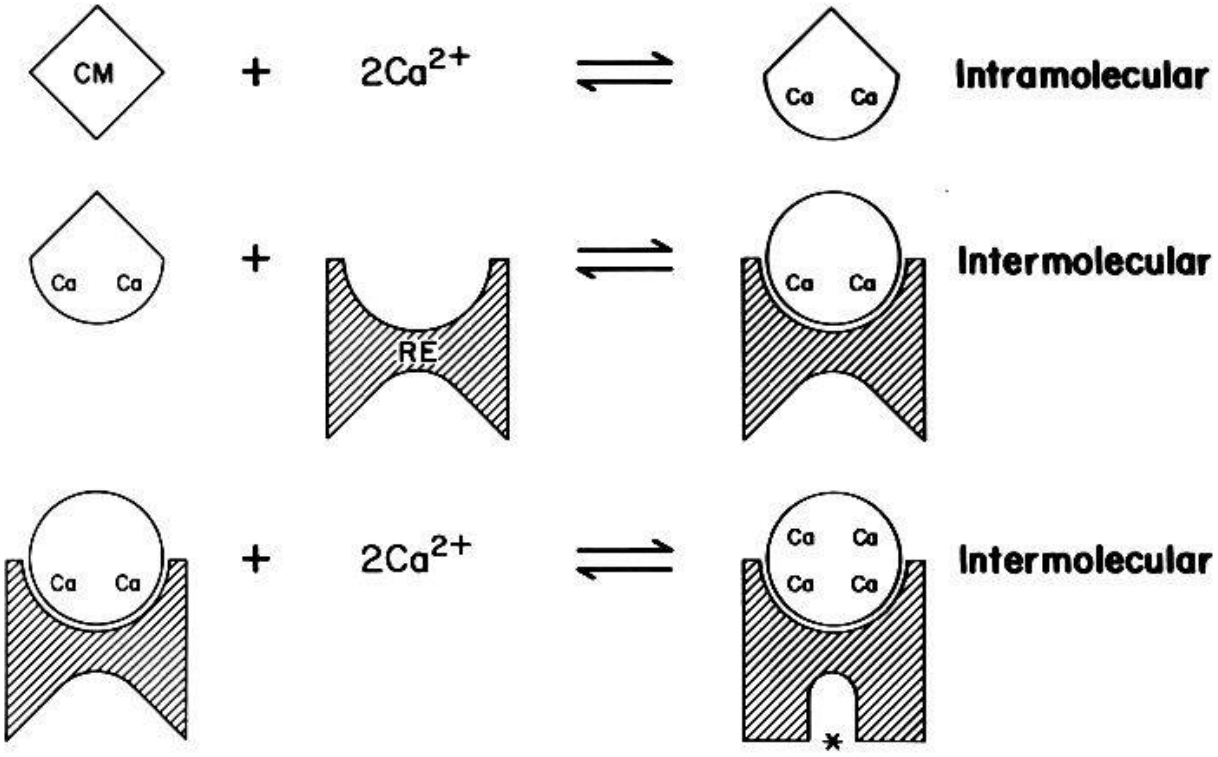

Fig. 6. A three step model of the binding reactions of calcium to calmodulin (CM) and of calmodulin to a response element (RE). Each change in shape is meant to represent a conformational change that enhances the binding of the next ligand in the sequence, or in the last step activates the response element. The sequence is an ordered one displaying highly positive cooperative interactions.

affinity of CM for the RE, another type of positive cooperativity. The third step is the binding of the last two calciums to calmodulin to produce the activated complex. These occur because the binding of RE to $Ca_2 \cdot CM$ causes a conformational change in CM which causes a marked increase in the affinity of the last two binding sites for calcium, a third example of positive cooperativity.

Because of the ordered nature of these reactions, once the system has been shifted to the activated state, it will remain in that state at a lower (Ca^{2+}) than that necessary to shift it into that state (Fig. 7). This property explains how a transient rise in $(Ca^{2+})_c$ leads to a sustained cellular response.

This property predicts that in order for the system to relax back into the non-activated state the $(Ca^{2+})_c$ must fall below its original or basal value. This is achieved by having the Ca^{2+}-Mg^{2+} ATPase (the calcium pump) of the plasma membrane regulated by calcium-cal-

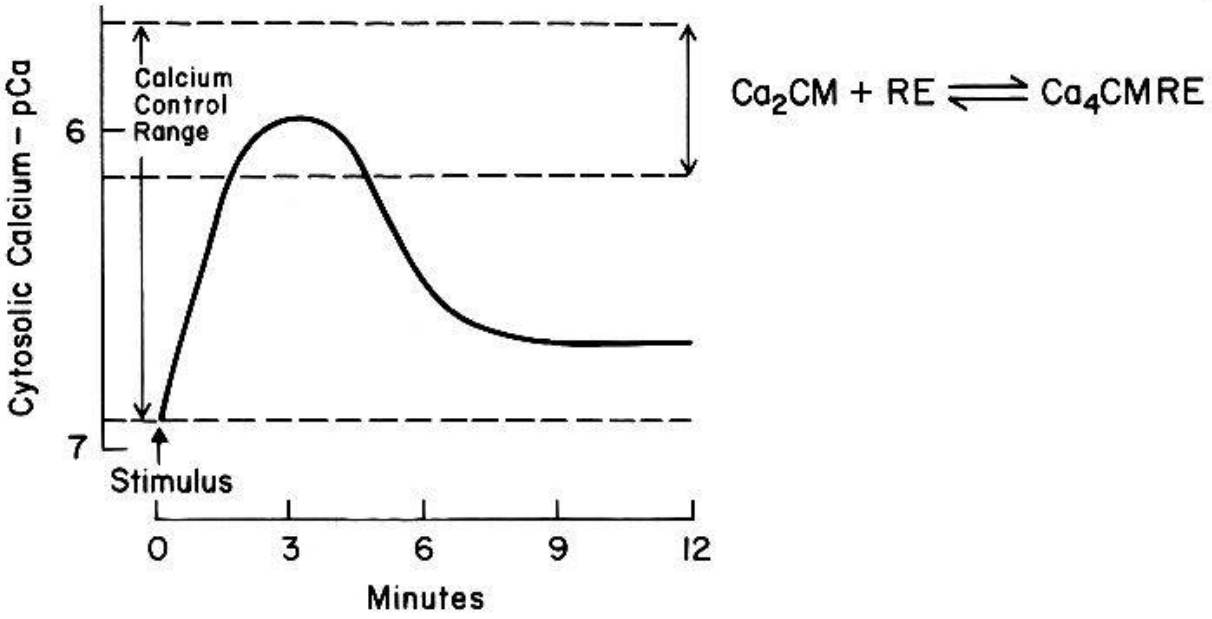

Fig. 7. A schematic illustration of activation of a response element within a cell induced by a transient rise in the calcium ion concentration of the cell cytosol.

modulin (36-38). In other words, the calcium pump is one of the calmodulin modulated response elements. This insures termination of response. However, in the situation in which a sustained response occurs, in order that termination does not take place there must be a sustained rate of calcium influx into the cell to balance the observed increase in efflux. The net accumulation of calcium by the cell is due to the fact that influx must be sufficient not only to balance efflux across the plasma membrane, but also the increased uptake of calcium by the mitochondria secondary to the slight increase in $(Ca^{2+})_c$. For example, if the $(Ca^{2+})_c$ in the activated cell is 0.4 μM, then the net accumulation of calcium by mitochondria would be approximately 1.3 μmoles/kg cell H_2O/min. The increased cycling of Ca^{2+} across the plasma membrane, and the increased net accumulation of calcium by cell and mitochondria are integral components of amplitude modulation in the calcium messenger system.

Even though the various components of amplitude modulation are integrated in a simple but elegant fashion, there is another means by which modulation can be achieved. This is what I have chosen to call sensitivity modulation (Fig. 3). Sensitivity modulation is by definition a situation in which a calcium-calmodulin modulated enzyme is activated by a change in the sensitivity of the system to activation by Ca^{2+}. The $(Ca^{2+})_c$ does not change, but more or less $Ca_4 \cdot CM \cdot RE^*$ is formed because of a shift in the calcium activation profile of a calmodulin modulated enzyme.

The activity of the enzyme phosphorylase b kinase, a calcium-calmodulin regulated enzyme, can be modulated either by a change in the amplitude of the calcium message, or by a change in its sensitivity to activation by Ca^{2+} (39-41). Such a change is brought about in the cascade of enzymes involved in regulating glycogenolysis in skeletal muscle when catecholamines act on this tissue. They cause a rise in (cAMP). This enhances the activity of the cAMP-dependent protein kinase. One of the substrates for this kinase is phosphorylase b kinase (Phos b kinase). This is a calcium-dependent protein kinase which has calmodulin as its regulatory subunit. When a subunit of Phos b kinase, other than calmodulin, is phosphorylated, the sensitivity of the enzyme to activation by Ca^{2+} increases, i.e., the K_m decreases from 3 to 0.5 uM (Fig. 8). This means that the enzyme becomes activated in a cell with a $(Ca^{2+})_c$ of 0.2-0.3 μM without a change in the concentration of $(Ca^{2+})_c$. This is an example of positive sensitivity modulation.

Negative sensitivity modulation is also achieved by the cAMP-dependent phosphorylation of calcium-dependent protein kinases. An example is myosin light chain kinase (Fig. 8). This is a calcium-dependent protein kinase in which calmodulin is a regulatory subunit (20, 21, 42-44). It serves in smooth muscle as the focus of Ca^{2+} action in regulating contraction. A rise in $(Ca^{2+})_c$ leads to an activation of the enzyme (K_m 0.8 μM). This activated enzyme catalyzes the phosphorylation of a light chain of myosin which in turn initiates the interaction between actin and myosin, i.e., contraction. In many smooth muscle cells, catecholamines acting via beta receptors cause relaxation (19). This effect is mediated by a rise in (cAMP). One of the consequences of the activation of the cAMP-dependent protein kinase is the phosphorylation of a subunit of myosin light chain kinase other than calmodulin (Fig. 8). This causes a shift in the sensitivity of this kinase (MLCK) to activation by calcium. (The K_m increases from 0.8 to 8 μM.) As a consequence, the activity of the enzyme (MLCK) is reduced. These types of sensitivity modulation mean that in a cell containing multiple calcium-calmodulin regulated enzymes, it is possible to alter the activity of one of these enzymes selectively. It also means that these response elements (enzymes) have different and variable sensitivities to activation by Ca^{2+}, so that the consequences (both qualitatively and quantitatively) of a given increase in $(Ca^{2+})_c$ will be different depending on the nutritional, and hormonal status of that particular cell. Because of this property, this rather sterotyped control device can elicit highly variable cellular responses: there is a considerable plasticity in the function of the calcium messenger system. The quite restricted range over which $(Ca^{2+})_c$ controls the functions of these various calmodulin-modulated response elements is the key to the resolution of our apparent paradox. The (Ca^{2+}) of the cell cytosol must be very precisely controlled, if $(Ca^{2+})_c$ is to serve as a messenger conveying information from cell surface to cell interior.

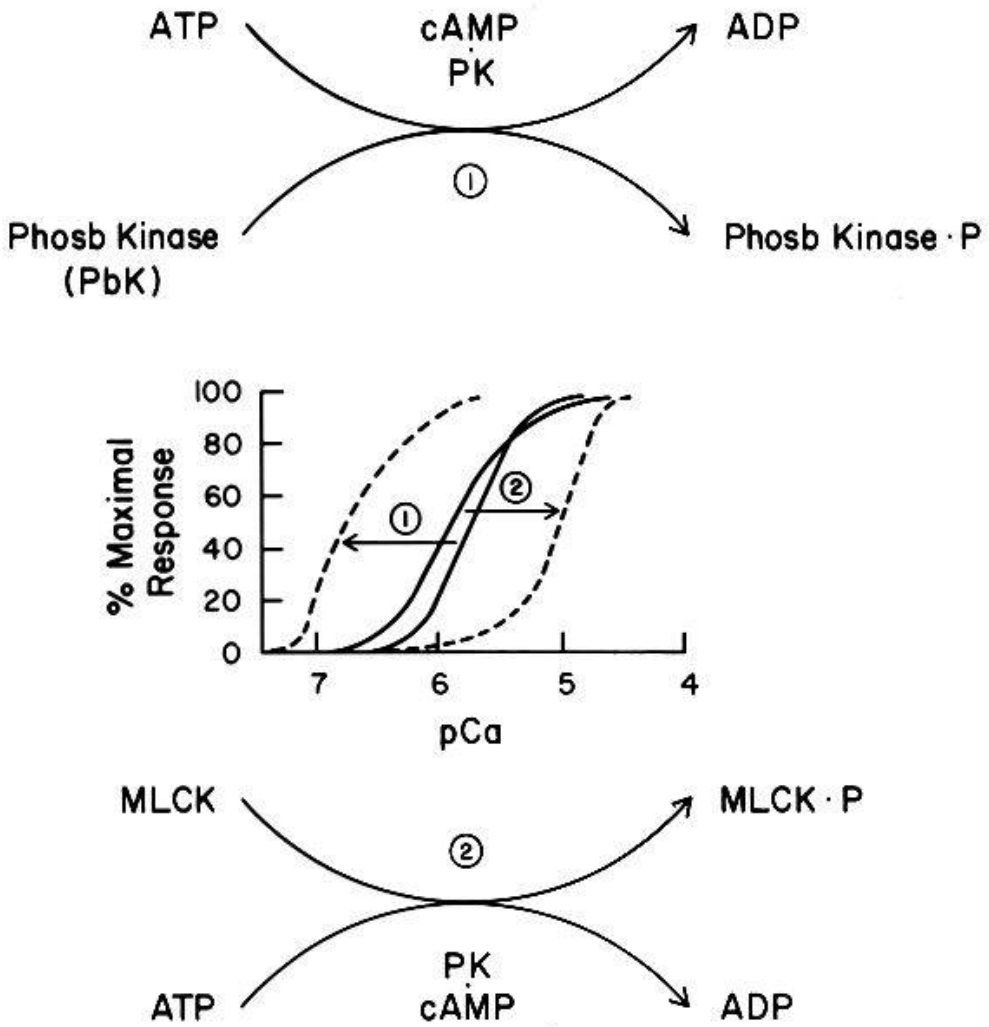

Fig. 8. A representation of the effect of the cAMP-dependent phosphorylation of PbK ① and MLCK ② upon the activation of each of these enzymes by calcium ion (center). Phosphorylation of PbK shifts its calcium activation curve to the left, i.e., it increases its sensitivity to activation by Ca^{2+}, and phosphorylation of MLCK shifts its activation curve to the right, i.e., it decreases its sensitivity to activation by Ca^{2+}.

These examples of sensitivity modulation in the Ca^{2+} messenger system resulting from changes in the cAMP messenger system are the most convincing molecular evidence supporting the concept of synarchic regulation. They provide an entirely new view of how cellular processes are regulated, and emphasize the importance of the Ca^{2+}-cAMP duality in stimulus-response coupling.

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