Calcium in the Regulation of Aldosterone Secretion and Vascular Smooth Muscle Contraction

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SUMMARY A model of angiotensin II action has been developed in which the flow of information from cell surface to cell interior proceeds by two temporally distinct branches: a calmodulin branch largely responsible for initiating the response; and a C-kinase branch for sustaining it. There are at least two initial events: a prompt and sustained increase in calcium influx rate, and prompt hydrolysis of phosphatidylinositol 4,5-bisphosphate. The latter leads to the generation of water-soluble inositol 1,4,5-trisphosphate and lipid soluble diacylglycerol. The rise in inositol 1,4,5-trisphosphate concentration causes the redistribution of intracellular calcium, a transient rise in the calcium concentration in the cytosol, and the activation of calmodulin-dependent enzymes, including protein kinase(s). As a result, several cellular proteins are rapidly phosphorylated and initiate the cellular response. The rise in calcium and these initial phosphorylation events are transient, however, so that an additional mechanism is necessary to sustain the response. The rise in diacylglycerol content, along with the transient rise in cytosolic calcium, leads to a shift of the C-kinase from a calcium-insensitive to a calcium-sensitive, plasma membrane-associated form. In this location, the activity of C-kinase is regulated by the rate of calcium flux across the plasma membrane. As a result of the activation of the C-kinase, a second set of cellular proteins becomes phosphorylated, and these control the sustained phase of the response. (Hypertension 10 [Suppl I]: I-23-I-26, 1987)

KEY WORDS • protein kinase C • phorbol esters • angiotensin II • adrenocorticotropic hormone • potassium

The generally accepted view of the messenger function of the calcium ion, Ca\(^{2+}\), is one in which a rise in the intracellular free calcium concentration evokes a response. When this model is applied to cells displaying a sustained response to a sustained presence of an extracellular messenger, the tacit assumption is that the extracellular messenger induces a sustained increase in the intracellular free calcium concentration and that this rise is sensed by a group of calcium receptor proteins that interact with other proteins, including protein kinases, to alter their function. In the case of vascular smooth muscle, this model has been extended to the molecular level by the further postulate that the sustained increase in free calcium concentration leads to sustained activation of a specific kinase, myosin light-chain kinase, thereby causing a sustained increase in the extent of phosphorylation of myosin light chain.\(^1\)\(^-\)\(^3\) However, neither assumption is correct. When angiotensin II (ANG II) acts on vascular smooth muscle or adrenal glomerulosa cells, only a transient increase in the free calcium concentration occurs.\(^4\)\(^-\)\(^5\) (W. Apfeldorf and H. Rasmussen, unpublished observations). This rise lasts 3 to 5 minutes in spite of the fact that contraction of the muscle or the secretion of aldosterone may continue for hours. Similarly, the extent of phosphorylation of myosin light chain rises promptly after agonist addition and then slowly declines. Within 20 to 30 minutes the phosphorylation of myosin light chain is back to its basal value in spite of a sustained contractile response.\(^6\)\(^-\)\(^7\)

In the course of investigating the mechanism by which this apparently transient calcium message leads to a sustained cellular response, we have developed a
Temporal Integration of Cellular Response

Our most extensive data come from studies with isolated bovine adrenal glomerulosa cells. When these cells are perfused with ANG II, the rate of aldosterone production undergoes a calcium-dependent, monotonic rise to a sustained plateau. Three observations indicate that no simple relationship exists between changes in calcium metabolism and cellular response. First, in cells exposed to ANG II, there is a very prompt rise in intracellular free calcium lasting only for 2 to 3 minutes. Second, in cells treated with a calcium ionophore (A23187), which induces a four-fold increase in calcium uptake rate, there is also a calcium-dependent increase in aldosterone production. The addition of ionophore, however, leads to only a transient increase in aldosterone production rate even though its effect on calcium influx rate is sustained. These two observations indicate that simply a change in calcium influx rate cannot lead to a sustained response. The third observation concerns the synergy between the effects of the ionophore and those of agents known to activate the enzyme, protein kinase C.

Based on these observations and a detailed analysis of the effects of ANG II on cellular calcium metabolism and protein phosphorylation, the following sequence of events has been postulated to occur in hormone-treated glomerulosa cells (Figure 1). Binding of ANG II to its receptor leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate, giving rise to two products: water-soluble inositol 1,4,5-trisphosphate (IP$_3$), and the lipid-soluble diacylglycerol (DG). Each serves a messenger function.

The rise in IP$_3$ induces the release of calcium from an intracellular pool (presumed to be in the endoplasmic reticulum), causing a transient rise in the free calcium concentration of the cell cytosol that activates a variety of calmodulin-dependent enzymes including calmodulin-dependent protein kinase(s). As a result, six cellular proteins become phosphorylated within 1 minute of ANG II addition, and cellular response is initiated. The rise in free calcium is transient, however, because the released calcium is rapidly pumped out of the cell. As a consequence, the phosphorylation of five of the six proteins is no longer increased 20 to 30 minutes after ANG II addition, when the free calcium is back to its basal level. The rise in DG content of the plasma membrane, together with the transient increase in cytosolic free calcium, causes a shift of protein kinase C (C-kinase) from its calcium-insensitive to its calcium-sensitive form. The latter form of the enzyme is thought to associate with the endoplasmic face of the plasma membrane. In this location its activity is controlled by the rate of calcium influx into the cell, which in turn is controlled by ANG II.

ANG II induces a sustained increase in the calcium influx rate that is matched by a sustained rate of calcium efflux, so that during the sustained response the cycling of calcium across the plasma membrane increases. The activity of C-kinase depends upon two factors: 1) the amount present in its membrane-associated form, and 2) the rate of calcium cycling. When both are increased, four proteins become phosphorylated and remain so throughout the sustained phase of the response. Of these proteins, one appears to be a common substrate for both the calmodulin-dependent kinase (active during the initial phase) and the C-kinase (active during the sustained phase), and three appear to be unique substrates of protein kinase C. These late phase phosphoproteins are thought to mediate the sustained phase of the response.

This temporal integration of events in the two branches of the calcium messenger system leads to the
observed integrated cellular response: a monotonic increase in the rate of aldosterone production rising to a plateau that is sustained as long as ANG II is present.

Our studies in vascular smooth muscle\textsuperscript{17, 18} are less extensive than those in the adrenal cell, but the model developed from the adrenal cell studies appears to be applicable to the smooth muscle. For example, the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) is able to induce a slowly developing, sustained, calcium-dependent contraction of vascular smooth muscle, and this contraction is rapidly and completely reversed by treatment of the muscles with forskolin, an activator of adenylate cyclase.\textsuperscript{26} Also, agents that alter plasma membrane calcium fluxes alter the time of onset or the rate of change of the contractile response to TPA. Furthermore, it has been shown that chemically skinned vascular muscle strips will display a calcium-dependent contraction in response to the addition of phorbol ester, and this contraction occurs in spite of the fact that no substantial change occurs in the extent of myosin light-chain phosphorylation.\textsuperscript{27}

In addition to these data, several other studies provide indirect support for the concept that C-kinase plays an important role in mediating smooth muscle contraction: 1) agonists stimulate polyphosphatidylinositol turnover in smooth muscle;\textsuperscript{28, 29} 2) addition of IP\textsubscript{3} to saponin-permeabilized, calcium-loaded smooth muscle causes a mobilization of calcium;\textsuperscript{30} and 3) C-kinase plays an important role in platelet activation,\textsuperscript{31} a process that has many similarities to smooth muscle contraction. Finally, our studies in another type of smooth muscle, tracheal smooth muscle, show that when carbachol induces a sustained calcium-dependent contraction, there is an activation of phospholipase C;\textsuperscript{32} a sustained increase in cellular DG content;\textsuperscript{32} and a changing temporal pattern of protein phosphorylation.\textsuperscript{30} Myosin light chain is phosphorylated initially and transiently, and desmin, synemin, caldesmon, and a group of low-molecular-weight cytosolic proteins, are subsequently phosphorylated and remain so during the tonic phase of contraction. These same late phase phosphoproteins are also seen in phorbol ester-treated cells.

Our present model of agonist-induced smooth muscle contraction is one in which agonists induce a dual flow of information from cell surface to cell interior: a calmodulin branch responsible for initiating contraction by activation of myosin light-chain kinase and the phosphorylation of myosin light chain, and a C-kinase branch responsible for sustaining the contractile response by the phosphorylation of cytosolic and intermediate-filament proteins. In the initial phase of the response, increases in IP\textsubscript{3} cause a release of intracellular calcium and a transient rise in cytosolic free calcium. This activates the calmodulin-dependent myosin light-chain kinase, resulting in the phosphorylation of myosin light chains and a rapid, initial contractile response. The rise in calcium is transient, however, and so the activation of myosin light-chain kinase is transient. In the sustained phase of the response, vascular tone is maintained by the flow of information through the C-kinase branch. The rate at which the C-kinase enzyme expresses its activity depends both upon the amount of the C-kinase enzyme that is in its activated, calcium-sensitive form, and the rate of calcium influx across the plasma membrane. In this view, the phosphoprotein products of the C-kinase are involved in regulating sustained contraction.

Modulation of Calcium Messenger System by the cAMP Messenger System

In spite of the apparent similarity of the organization of the calcium messenger system in these two tissues, their responses to an increase in cyclic adenosine 3', 5'-monophosphate (cAMP) content are quite different. In the case of the adrenal cells, addition of either adrenocorticotropic hormone (ACTH) or high potassium (8 mM) leads to activation of adenylate cyclase and a rise in cAMP content (ACTH has a greater effect on cAMP content than K\textsuperscript{+}).\textsuperscript{13, 14} Each of these agents also increases the calcium influx rate (K\textsuperscript{+} has a greater effect than ACTH) and transiently increases the free calcium concentration of the cell. Neither increases the hydrolysis of inositol phospholipids or activates the C-kinase branch of the calcium messenger system. Nonetheless, both high potassium (8 mM) and ACTH induce a rapid and sustained increase in the rate of aldosterone secretion.\textsuperscript{11, 12} The two messengers, calcium and cAMP, act synergistically to enhance the aldosterone secretory response without activating the C-kinase pathway. Similarly, if cells are exposed to forskolin, an activator of adenylate cyclase along with A23187, a calcium ionophore, a submaximal aldosterone secretory response is induced that is enhanced by agents that increase plasma membrane calcium influx.\textsuperscript{14} One can view the synergism in the action of calcium and cAMP in terms of cAMP altering in a positive way the set point around which the messenger calcium operates. Hence, in the case of both ACTH and potassium action, the increase in cAMP acts as a positive sensitivity modulator\textsuperscript{33} of messenger calcium. From a practical point of view, this means that the effect of the calcium message in the adrenal glomerulosa cell can be enhanced and its effects prolonged by either of two mechanisms: activation of the C-kinase enzyme or increase in the sensitivity of calmodulin-dependent, calcium-regulated enzymes to the effects of calcium.

The situation in vascular smooth muscle is quite different. Agents that induce an increase in cAMP content inhibit the contractile response to agonist,\textsuperscript{34, 35} or to agents that bypass receptor-mediated events and activate calcium influx and C-kinase directly.\textsuperscript{17-20} In this tissue, a rise in cAMP alters in a negative manner the set point around which the calcium messenger system operates. The molecular mechanism by which this is mediated is not known.

Conclusion

These new insights into the organization of the calcium messenger system in adrenal glomerulosa and vascular smooth muscle cells have implications for our current views of the pathogenesis of hypertension. In particular, our present model of how tone is main-
Maintained in vascular smooth muscle is radically different from the currently accepted one. In our model, events occurring almost exclusively at the plasma membrane determine muscle tone during the sustained phase of the response. The two major events are 1) the amount of activated C-kinase associated with this membrane, and 2) the rate of calcium influx (or more correctly, perhaps, the rate of Ca\(^{2+}\) cycling) across the plasma membrane.\(^{10,14}\) Hence, agents that alter either calcium influx or efflux rates will alter tone not by influencing the calcium concentration in the bulk cytosol but by influencing the calcium concentration in a subplasma membrane domain. Furthermore, since both the activation and activity of the C-kinase are dependent in part upon the lipids with which the enzyme associates, this new model provides a possible direct link between membrane lipid composition and vascular tone.

Finally, our model predicts that one or more cytosolic proteins, which are substrates for protein kinase C and that undergo phosphorylation when this enzyme is activated, are the messengers carried from cell surface to the contractile system. Defining the number and nature of these proteins and their specific roles should provide new insights into the molecular mechanisms by which a sustained contraction of vascular smooth muscle is achieved.

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