VASCULAR NEUROEFFECTOR MECHANISMS

edited by J. A. BEVAN, T. GODFRAIND,
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PREFACE

The neurovascular junction plays a strategic role in the regulation of blood pressure and regional circulation. The vessel wall being alive and complex, its motility status is the result of a constant interaction of intravascular (nerve transmitters, endothelial factors) and extravascular agents (hormones and drugs) with the smooth muscle cells. The excitability of these cellular elements is dependent on the regulation of transmembrane ionic movements, resulting from the opening and closing of channels and the action of energy driven pumps. Recent results presented in this symposium show that vascular smooth muscle excitability and contractility is modified by hormones, drugs, and newly described endogenous substances (digitalis-like factor, peptides of atrial origin) which are active in modifying salt and water metabolism.

The initial part of this volume deals with actual trends in knowledge of excitation-contraction coupling and contraction in vascular smooth muscle. Following chapters discuss the localisation and subtypes of receptors in different territories, the role of endothelium in vascular relaxation as well as the structure and function of smooth muscle and endothelial cells in culture, and some new drugs acting on vascular smooth muscle. Subsequent sections deal with the mechanism of vasospasm in cerebral and coronary circulation, the role of dopamine and epinephrine in peripheral control of blood pressure, and on new techniques used in the assessment of vascular smooth muscle excitation and contraction.

These proceedings should give an up to date image of recent investigations and discoveries in vascular pharmacology and physiology. They will therefore be of great interest to all those involved in work in basic or applied research in biochemistry, pharmacology, physiology, physiopathology and medicine of the cardiovascular and cerebrovascular systems.

M. Worcel

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CONTENTS

CONTROL OF EXCITATION-CONTRACTION COUPLING AND CONTRACTION IN VASCULAR SMOOTH MUSCLE

Vascular Neuroeffector Mechanisms, J.A. Bevan et al. (eds.)

WHAT REGULATES ACTIVATOR CALCIUM?

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For more than two decades a headline item in the narrow world in which we operate has been the relationship between the calcium ion and vascular smooth muscle contraction. From early on we have known that the intracellular concentration of calcium is the major determinant of this muscle's contractile activity. Hence, this calcium is referred to as "activator calcium". For those of us who feel the responsibility of knowing what makes this muscle contract and relax, investigation into the regulation of this calcium concentration is of prime importance. Our knowledge has been increasing with a Q 10-years of at least three. But as our knowledge has increased, so has our recognition of the complexity of the regulatory process. Not only is the regulation of the concentration of calcium complex, but the actions of calcium itself as they affect the concentration of activator calcium are numerous and important. The regulation of the concentration of activator calcium is truly a bewildering labyrinth of interacting variables.

We will not try to straighten out this mess. What we will do is, first, paint with a broad brush the overall picture of the regulation of the concentration of activator calcium as it looks in mid-1984. Then we will describe some of the ways in which this omnipotent cation regulates its own concentration. References have been selected from which the diligent reader may get more references.

THE OVERALL PICTURE

Before considering the specific factors that raise and lower the concentration of activator calcium (see Figure 1) we should make inquiry as to the relevant calcium concentrations involved, and question whether there are factors other than the calcium concentration that influence the contractile state of the muscle.

Studies by Rüegg and Paul[1] have contributed answers to both of these questions. In "chemical skinned" preparations of small fibers from the pig carotid, known concentrations of calcium in the muscle bath have access to the intracellular contractile machinery. This calcium activates the enzyme myosin light chain kinase (MLCK) which phosphorylates the myosin light chain to initiate contraction. Half-maximal contraction of these fibers occurred with a calcium concentration of 10^{-6}M. Calmodulin (4×10^{-6}M) increased the sensitivity to calcium so that the ED_{50} was lowered to 10^{-7}M (see Figure

2). Since the intracellular concentration for calmodulin is 5 x 10^{-6}M, the latter sensitivity is relevant to the in situ process. Murphy and his colleagues[2] have described a persistent stress maintenance by vascular smooth muscle called a latch state. This state does not require myosin light chain phosphorylation, has a very low energy cost and a low calcium requirement. Its molecular mechanism is not known.

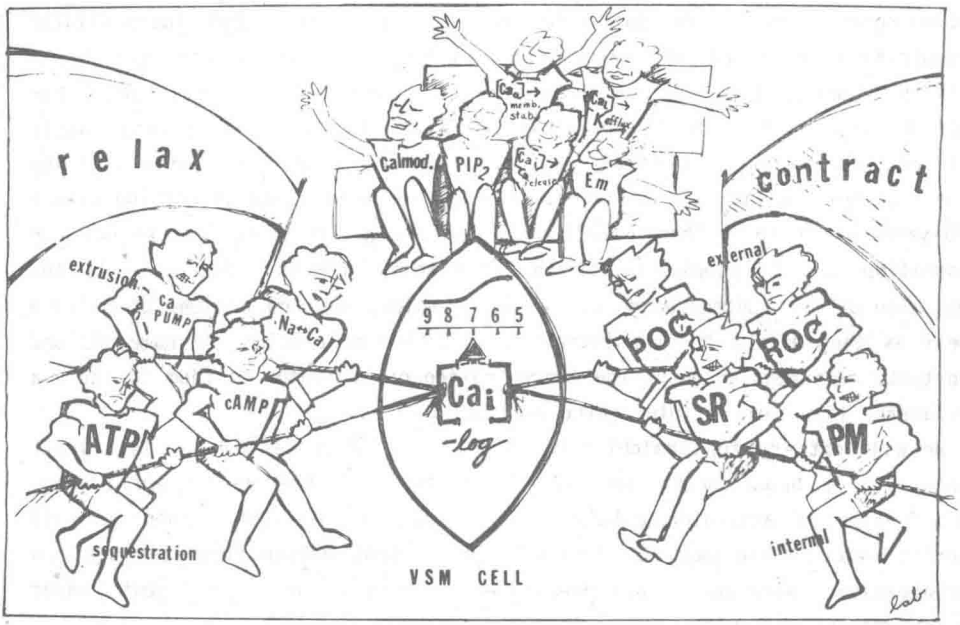

Fig. 1. Forces that pull the concentration of activator calcium up or down and factors that cheer them on (see text for clarification).

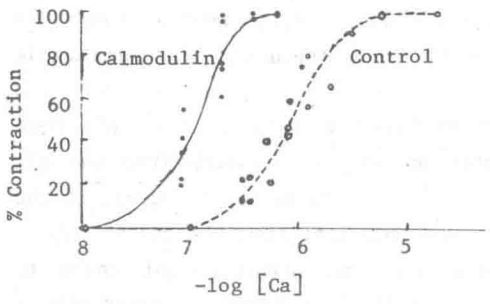

Fig. 2. Effect of calmodulin on calcium responsiveness of hog carotid skinned smooth muscle fibers. Force is plotted in relation to calcium concentration (M) in presence of calmodulin (filled circles) and in the absence of calmodulin (open circles). The results obtained from several skinned carotid artery strips were normalized with respect to maximum active force. (From reference 1, by permission.)

Cyclic AMP-dependent protein kinase (cPK) is known to phosphorylate MLCK and thereby to decrease its activity.[3] Rüegg and Paul[1] also observed that cPK depressed the contractile response of their skinned carotid fibers, especially the response elicited by low concentrations of calcium. The action of cAMP in this catalytic subunit establishes it as a possibly important contender for a regulatory (relaxant) role in the vascular smooth muscle contractile machinery. This issue is by no means settled. Jones et al.[4] have presented evidence that the primary relaxant activity of cAMP depends on an unrelated action that it has on the plasma membrane. Saida and van Breemen[5] found no effect of cAMP on calcium-induced contraction of chemically skinned fibers from the mesenteric artery. They report that cAMP augments calcium-induced calcium release. These mechanisms suggest that the effects of cAMP on vascular smooth muscle are indirect and mediated through changes in calcium concentration. Of course there must be sufficient ATP present to supply energy for the contractile process but a variation in this energy source does not normally serve as a regulator of vascular smooth muscle contraction.

The complexity of the overall picture of the regulation of activator calcium concentration is exacerbated by the variability of this process that occurs with the site of origin of the blood vessel, with the age, sex and species of the animal and with different agonists. These variabilities make major contributions to the volume of literature on the subject.

The Contraction Team

This team is comprised of players that increase the concentration of activator calcium. The source of this calcium is either from the extracellular space or from cellular sites in which calcium is sequestered.

External Sources. A very important member of this team is the potential operated channel (POC). It opens permitting the inflow of extracellular calcium when the transmembrane potential decreases and it closes when the potential increases. The membrane potential of vascular smooth muscle is depolarized by either action potentials or graded depolarization. Because so many physiological and pharmacological interventions alter membrane potential, this is a very commonly used system for regulating activator calcium concentrations. The magnitude of the membrane potential is determined by both diffusion potentials and by an electrogenic pump activity.

The potassium diffusion potential is a major contributor to the membrane potential, hence the POC is readily studied by increasing extracellular potassium concentration. This intervention decreases the potassium gradient and membrane potential, thereby opening the POC to increase the concentration of activator calcium. The type of channel opened by membrane depolarization is the one most readily blocked by calcium channel blockers.[6] Högstätt and

Andersson[7] have presented evidence which they interpret as indicating there are two separate POC's: one that opens rapidly and transiently and one that opens slowly and stays open. They hypothesize that the rapid channel is inactivated by calcium binding to the channel. An increase in intracellular calcium concentration may also close POC's by activating potassium efflux channels (see below).

The second generator of the membrane potential is the electrogenic pump driven by Na/K-ATPase. Interventions that increase its activity cause hyperpolarization with a resultant closure of POC, and vice versa. Monensin, a sodium ionophore, increases intracellular sodium concentration which stimulates the pump. This hyperpolarizes the muscle and depresses the contractile response (see Figure 3). When ouabain is used to turn off the pump this depression does not occur.[8]

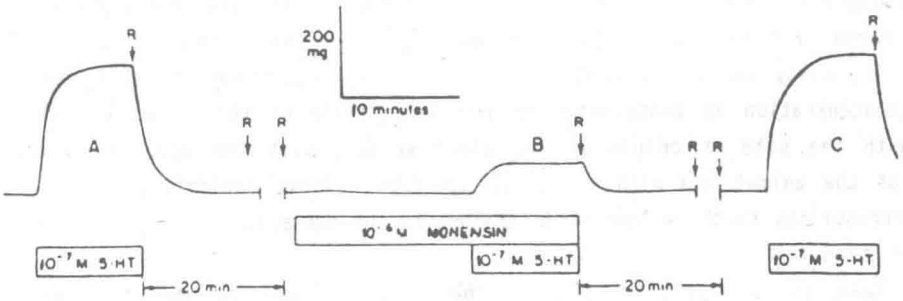

Fig. 3. Monensin and contractile responses to serotonin (5-HT) in dog coronary artery. Monensin, a sodium ionophore, inhibits contractile responses to serotonin in dog coronary artery. The inhibition by monensin is reversed by treatment with ouabain (10^{-5}M). (From reference 8, by permission.)

A similar mechanism probably accounts for the unexpected finding that following treatment of vascular smooth muscle with serotonin, and after this agonist has been washed from the bath there is a half hour period during which the muscle is hyporesponsive to any agonist (see Figure 4).[9] This attenuation is prevented by ouabain and does not occur when the system has been pretreated with verapamil. We conclude that serotonin treatment has permitted an increase in intracellular sodium concentration, that this is driving the pump to produce hyperpolarization which closes the POC. If POC are already closed by verapamil, the effect of hyperpolarization is not evident. Further documentation for this mechanism of the post serotonin attenuation was observed in the depression of the stimulated calcium influx observed during this period (see Figure 5).

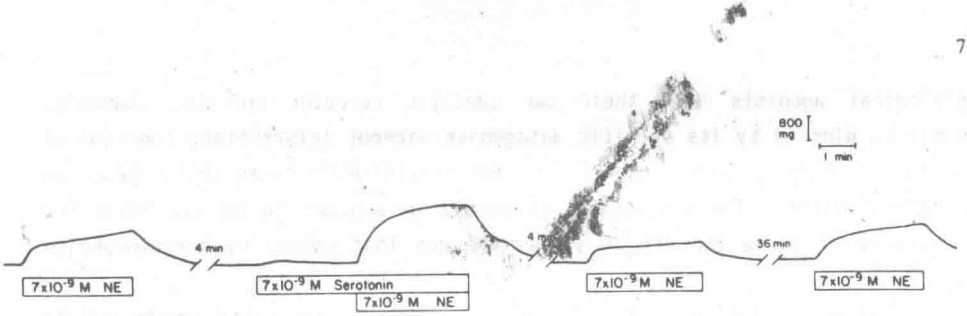

Fig. 4. Serotonin attenuation of contractile responses to norepinephrine in dog mesenteric artery. Treatment with serotonin (7×10^{-9}M) potentiates contractile responses to norepinephrine (7×10^{-9}M NE). Following this treatment, contractile responses to norepinephrine are depressed compared to initial responses. This attenuation is blocked by ouabain. (From reference 9, by permission.)

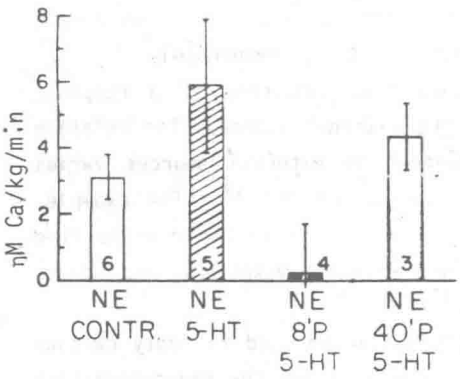

Fig. 5. Effect of serotonin (5-HT) on norepinephrine (NE) stimulated calcium influx in dog mesenteric artery. Calcium influx in the presence of 5-HT was greater than control (CONTR). Eight minutes after 5-HT had been rinsed from the bath the NE stimulated influx was reduced to near zero but had returned to control levels in 40 minutes. (From reference 9, by permission.)

The importance of the electrogenic pump as a determinant of membrane potential and hence of the degree of opening of the POC is evident in observations that it contributes to relaxation produced by ß-adrenergic activation, cAMP[10] and prostaglandins[11].

Calcium may also enter the cell by a channel that is not influenced by the membrane potential. These channels are opened by specific agonists' occupancy of receptors, hence are called receptor operated channels (ROC). Norepinephrine operates such a channel. One characterization that differentiates the ROC from the POC is that the POC is much more readily blocked by calcium entry blockers. Thus when vascular smooth muscle is treated with nifedipine, the contractile response to KCl depolarization may be negligible. Yet stimulation of this nifedipine-treated, depolarized muscle with norepinephrine will still cause a large contraction.[6] Here the POC's have been blocked, the membrane potential has been locked by depolarization, and the ROC's have been opened with norepinephrine. Many important

physiological agonists have their own specific receptor operated channels. Each may be blocked by its specific antagonist without interrupting function of the others. There is some evidence for individualities between ROC's opened by different agonists. The ROC opened by serotonin appears to be available for the entrance of large amounts of sodium whereas that opened by norepinephrine is not.[9]

In addition to POC and ROC, calcium may enter the vascular smooth muscle cell by a "leak channel".[12] These channels may be increased in hypertension and be responsible for an increase in vascular smooth muscle tone.[13]

Internal Sources. A large amount of calcium is bound to or stored in the sarcoplasmic reticulum, the plasma membrane and the mitochondria. Calcium in the mitochondria apparently does not play an important role as a source of activator calcium. Calcium from the other two storage sites does play a role in the contractile response to most agonists. It is responsible for the initial rapid response whereas the maintained slow component of a response results from activator calcium arising from the external source. The relative amount of calcium coming from internal compared to external sources varies greatly depending on the source of the vascular smooth muscle. For example, that from the aorta derives a large portion of its activator calcium from internal sources whereas the activator calcium for small mesenteric resistance vessels arises largely from external sources.[14]

Because caffeine in high concentration (25mM) can be used to empty calcium from the sarcoplasmic reticulum it has been used to study the characteristics of this calcium pool.[15] The pool of calcium that can be released by caffeine has been found to be different from that releasable by norepinephrine stimulation. Thus, after the norepinephrine-sensitive calcium pool has been completely emptied, residual calcium can be released by caffeine. There is evidence indicating that the norepinephrine-sensitive store of calcium is closer to the cell surface than is the caffeine-sensitive store. The involvement of the membrane as a storage site for agonist-releasable calcium has been inferred from studies by Loutzenhiser and van Breemen.[16] They observed that the prostaglandin analogue U-44069 had a stimulating effect on calcium influx only after calcium had loaded this site for at least two and a half minutes (see Figure 6).

Calcium may be released from the sarcoplasmic reticulum by one or more of several methods: 1) depolarization of the sarcoplasmic reticulum membrane; 2) an increment in intracellular calcium concentration; and 3) some action of polyphosphoinositides (see below).

Relaxation team

Paradoxically, this "relaxation" team in reducing the concentration of