This brief review of the rapidly developing research on vascular smooth muscle presents the state of the art as I see it from within my own frame of reference. For a more objective, detailed insight into the workings of vascular smooth muscle, several substantial reviews and compendiums may be read (1-7).

**CONTRACTILE PROTEINS**

The mechanical events responsible for the contraction of vascular smooth muscle are associated with its contractile proteins. These proteins not only develop the mechanical force responsible for the contraction but also act as the enzyme that catalyzes the release of energy by which this force is developed. They are both the spark plug and the piston of the contractile machine.

The contractile proteins of vascular smooth muscle are arranged in well-organized thick and thin filaments (8-10). The thick filaments, presumably bundles of myosin molecules, average 15.5 nm in diameter and have lateral projections suggestive of cross-bridges extending toward adjacent thin filaments. The thin filaments, presumably fibrous actin, average 5-8 nm in diameter and appear to be attached to dense bodies that are usually connected to the cell membrane. Contraction of vascular smooth muscle most probably is effected by some version of the Huxley sliding filament mechanism.

The most easily interpretable studies of the functions of the contractile proteins are those performed in isolation with the determinants of the enzymatic and physical responses tightly controlled. There is a qualitative similarity between the actomyosin of vascular smooth muscle and the actomyosin of skeletal muscle (11) evidenced by the observation that a hybrid actomyosin can be prepared by combining myosin from one of these types of muscle with actin from the other; this hybrid provides a functionally active enzyme. Although the adenosinetriphosphatase (ATPase) activity of skeletal muscle actomyosin is many times faster than the activity of vascular smooth muscle actomyosin, the speed of activity of the hybrid preparation is determined by the source of the myosin. This observation correlates with the extensive studies by Bárány (12) showing that a direct parallel exists between the maximum velocity of shortening of a muscle and the ATPase activity of its actin-activated myosin. These observations bear the important implication that the shortening velocity of vascular smooth muscle has the actomyosin ATPase activity as its rate-limiting factor. This slow release of chemical energy is reflected in the slow physical changes in the actomyosin molecule observed in superprecipitation studies (13) or in studies of contraction velocity of glycerinated fibers (14).

All the indexes of functional activity of native actomyosin from vascular smooth muscle (ATPase activity, superprecipitation, and contraction of glycerinated fibers) have the same low requirement for activator calcium. Half-maximal activity of any of these processes occurs at an ionic calcium concentration of about 10⁻⁶ M (13). The parallel between the calcium requirement for the enzymatic activity and that for the physical change strongly indicates that the physical change is directly dependent on the enzymatic activity which releases energy from ATP. This calcium concentration is also the concentration required for the activation of the native actomyosin of skeletal muscle. Ebashi and Endo (15) showed that at a calcium concentration of 10⁻⁶ M half of the troponin of native actomyosin from skeletal muscle has bound calcium; therefore, it may be assumed that calcium combines with troponin in vascular smooth muscle as it does in skeletal muscle. The troponin-tropomyosin system, which normally inhibits the ATPase activity of actomyosin, no longer inhibits this activity when
calcium is combined with troponin; thus, the actin-activated myosin releases energy from ATP for contraction. This role of calcium in the contractile process of vascular smooth muscle is partially confirmed by the observation that, in storage at 4°C, native actomyosin from vascular smooth muscle loses its calcium requirement for activation; the troponin-tropomyosin system is labile and, with aging, is no longer capable of inhibiting the ATPase activity of actomyosin (13).

The critical dependence of activation of native actomyosin on calcium provides for the dependence of activation of contraction in the intact smooth muscle cell on an increase in intracellular calcium concentration (activator calcium). Other factors acting at the level of the contractile proteins, however, have not been completely eliminated, at least as secondary contributors to vascular smooth muscle contraction. The magnesium concentration required for activation of native actomyosin of vascular smooth muscle is 10–100 times greater than the concentration required for activation of actomyosin from skeletal or cardiac muscle (16). ATPase activity is inversely related to the ionic strength to which the actomyosin is exposed (17). This relationship may be somewhat responsible for the relaxation of vascular smooth muscle in hypertonic solutions. Because there is conflicting evidence (17, 18) about the influence of the sodium ion on the functional activity of native actomyosin from vascular smooth muscle, it is not possible to determine whether sodium produces an effect on vascular smooth muscle contraction by an action at this level.

In addition to myoplasmic calcium concentration, the fiber length of vascular smooth muscle is a physiological determinant of the force developed by its contractile proteins. This relationship is similar to the length-work curve that forms the basis for the Frank-Starling mechanism of the heart. Speden and Freckelton (19) observed that, for the central ear artery of the rabbit, the peak of the length-work curve occurs when the artery is stretched by a transmural pressure of 113 mm Hg.

**EXCITATION-CONTRACTION COUPLING**

Since contraction of vascular smooth muscle is initiated by extremely low calcium concentrations, it is important to explore the source of this activator calcium and the mechanism of its release into the environment of the contractile proteins. Figure 1 illustrates schematically the possible systems involved in the alterations of intracellular calcium concentration that regulate contraction and relaxation of the muscle. The processes that increase intracellular activator calcium concentration are represented by thick arrows, and the processes that decrease intracellular activator calcium concentration are represented by thin arrows. Some or all of these processes probably occur continuously, and the concentration of activator calcium may be expressed as the difference between the rates of these processes that result in accumulation and those that result in depletion of intracellular calcium (according to the relationship expressed at the bottom of the figure). The intracellular calcium concentration

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[Ca^{2+}] = (a + b + c + d) - (a' + b' + c' + d')
\]
may be increased to a level that will activate the contractile proteins either by increasing the rates of accumulation or by decreasing the rates of elimination or sequestration of calcium.

Activator calcium may come from either intracellular or extracellular sources, and alpha-adrenergic stimulation uses calcium from both sources. An initial fast response appears to be caused by calcium of intracellular origin, and a maintained slow response appears to be caused by calcium from the extracellular pool (20–22). In the resting muscle the concentration of activator calcium in the myoplasm is less than $10^{-7}M$, although the concentration of calcium in the extracellular pool is greater than $10^{-3}M$. Thus, for vascular smooth muscle to be relaxed, its plasma membrane must support a 10,000-fold concentration gradient of the calcium ion.

There are three candidates for the site of the cellular calcium pool—sarcoplasmic reticulum, mitochondria, and plasma membrane with its surface vesicles. In recent years three types of studies have furnished evidence about these possible sources of activator calcium: (1) electron microscope studies, (2) cell fragment studies, and (3) studies employing calcium-blocking agents.

Recent electron microscope studies by Devine et al. (23) have demonstrated that the sarcoplasmic reticulum occupies an appreciable part of the vascular smooth muscle cell, ranging from over 5% of the volume in the aorta and the main pulmonary artery to approximately 2% in the portal anterior mesenteric vein and the mesenteric artery. These authors made the interesting corollary observation that smooth muscle containing the larger amounts of sarcoplasmic reticulum maintains its contraction better in a calcium-free environment than does muscle with relatively little intracellular sarcoplasmic reticulum. They showed that the extracellular markers, ferritin and lanthanum, do not enter the sarcoplasmic reticulum but that the sarcoplasmic reticulum does accumulate the bivalent cation marker, strontium. These authors also observed that the mitochondria of the vascular smooth muscle cell accumulate barium and strontium and, therefore, are possible sequestration sites for the bivalent cation calcium. Both the sarcoplasmic reticulum and the mitochondria make close contact with the plasma membrane and the surface vesicles. The latter are differentiated from the sarcoplasmic reticulum by the fact that they are open to the extracellular markers.

Recent studies (24, 25) have shown that both mitochondrial and microsomal fractions from vascular smooth muscle are capable of energy-dependent sequestration of calcium. Study of the uptake and the release of calcium from these cell fragments promises valuable insight into the details of the control of activator calcium, since, for instance, angiotensin in physiologically active concentrations accelerates the release of bound calcium from the microsomal fraction (25).

Agents which effectively decrease membrane permeability to calcium have been valuable tools for studying the source of activator calcium. At least four such agents have been used recently: cinnarizine (26), SKF 525A (27), verapamil (28–30), and lanthanum (31). Vascular smooth muscle incubated in a calcium-free potassium sulfate depolarizing solution can be caused to contract by the addition of low calcium concentrations to the muscle bath. This response presumably occurs because the added calcium passes through the membrane which has been made highly permeable by the potassium sulfate–induced depolarization. Various calcium-blocking agents eliminate or greatly reduce this contractile response. These agents are much less effective in eliminating the response produced by agonists such as epinephrine, angiotensin, or histamine, suggesting that the contractile responses initiated by these agonists are less dependent on the passage of extracellular calcium through the plasma membrane than is the contractile response initiated by potassium-induced depolarization.

van Breemen et al. (31) demonstrated that lanthanum blocks the passage of calcium across the cell membrane of vascular smooth muscle and that it replaces all calcium bound to extracellular structures. By measuring the calcium remaining in segments of rabbit aorta, these researchers estimated intracellular calcium content. Using this technique, they observed that there were parallel rates of increase in tension and in intracellular calcium content in response to activation by potassium-induced depolarization or by lithium substitution for sodium. However, norepinephrine ($10^{-6}M$) caused a maximum increase in tension with no increase in intracellular calcium concentration; which again indicates that the activator calcium involved in contraction in response to alpha-adrenergic stimulation must have its origin primarily in an intracellular pool. When this pool is discharged by norepinephrine, histamine or angiotensin after the muscle has been in a calcium-free lanthanum-containing medium for 30 minutes, a single contraction occurs, but the muscle will not
respond subsequently to stimulation with any of the three agonists. Therefore, each of the three agents appears to use the same calcium pool. The relative significance of the plasma membrane, the sarcoplasmic reticulum, and the mitochondria as sources of calcium for the physiological response to these agents needs clarification.

Recognizing that the degree of contraction is determined by the concentration of activator calcium in the environment of the contractile proteins and that this calcium concentration reflects the difference between the rate of release of ionized calcium into the myoplasm and its rate of sequestration or extrusion, we still need information about the molecular mechanisms that regulate these movements of calcium and the means by which vasoactive agents influence these mechanisms. Probably the most significant hiatus in our understanding of the contractile machinery of vascular smooth muscle is the mechanism by which norepinephrine causes a release of activator calcium from the cellular sites where it is sequestered. Some level of satisfaction, but without adequate experimental support, may be derived from the hypothesis that a constrictor agonist may release calcium from the surface vesicles, as demonstrated by Baudouin et al. (25) for angiotensin, and that this calcium may act as “trigger calcium” (32) for the regenerative release of calcium from the sarcoplasmic reticulum (33).

The role of cyclic adenosine monophosphate (AMP) in the response of vascular smooth muscle to adrenergic and other stimuli is a matter of current interest but little knowledge. There is evidence that the content of cyclic AMP increases with beta-adrenergic activity (34). Furthermore, the observation that exogenous dibutyl cyclic AMP can mimic the effect of beta-adrenergic activation indicates that this increase may be an essential step in calcium sequestration and, hence, in relaxation. No such relationship has been identified between cyclic AMP and alpha-adrenergic activity.

ELECTRICAL AND NONELECTRICAL ACTIVATION

The contraction of vascular smooth muscle may be initiated by either electrical or nonelectrical activation. The classical model used for study of electrical activation is the portal vein. Smooth muscle in this vessel has spontaneous action potentials which cause the delivery of activator calcium into the myoplasm, and, hence, cause contraction of the muscle. However, this muscle and other vascular smooth muscle can be made to contract in response to norepinephrine and other physiological agonists when the cell membrane has been completely depolarized by isotonic potassium sulfate. Under these conditions, the response to the constrictor agonist occurs in the absence of a change in membrane potential and, therefore, is reasonably called nonelectrical activation. The unanswered question is whether the all-important vascular regulatory effect of norepinephrine that occurs in vivo is effected by electrical or nonelectrical activation.

Recent microelectrode studies support the possibility that norepinephrine usually causes contraction of smooth muscle in large arteries without the occurrence of action potentials. Haeusler (28) reported that in over 200 microelectrode penetrations of at least 10 seconds each in the smooth muscle cells of the rabbit pulmonary artery contracting in response to norepinephrine \(10^{-6}\)M, he observed no action potentials. He did find that norepinephrine reduced the membrane potential from a mean of 58.4 mv to 43.5 mv. Mekata and Niu (35), using lower concentrations of epinephrine on the common carotid artery of the rabbit, observed contraction of this vascular smooth muscle without action potentials or a change in membrane potential.

These more recent microelectrode studies agree with the earlier observation by Somlyo and Somlyo (36) that the same pattern of inequality of maximal responses to epinephrine, angiotensin, and vasopressin is obtained whether the smooth muscle is polarized or depolarized. This observation indicates that the inequalities in response in the polarized state are not due to differences in the electrical phenomena of the membrane and that factors which determine the magnitude of nonelectrical activation are important when the cell is polarized. There is no evidence that the pathway for nonelectrical activation in the depolarized state is turned off when the cell is polarized. Therefore, although one must always reckon with the enormous difficulties associated with microelectrode studies in the blood vessel wall, it seems safe to conclude that the important physiological constrictor agents are capable of initiating an increase in activator calcium concentration not dependent on action potentials. It is possible that most regulation of vascular smooth muscle effected by these agents occurs in situ by the nonelectrical activation pathway.
Possibly related to the role of electrical properties of the membrane in excitation are minor changes in the concentrations of extracellular sodium (37) or potassium (38) that produce important changes in vascular smooth muscle contraction and relaxation. These changes are complex, and the mechanisms by which the monovalent cations produce their effects are conjectural. An increase in sodium concentration appears to enhance a contraction caused by a release of sequestered calcium but to depress a contraction caused by an influx of extracellular calcium (20). The mechanisms that may account for these effects of sodium are (1) the competition between sodium and calcium for membrane transport or sequestering sites, (2) the sodium-calcium transmembrane exchange systems, and (3) the influence of the transmembrane concentration gradient of sodium on resting membrane potential and, hence, on membrane excitability.

The effects of changes in extracellular potassium concentration are similarly complex. Exposure to a potassium-free medium leads to a loss of responsiveness that has been attributed to a disturbance in the normal release of calcium in excitation-contraction coupling (39). Increases in potassium concentration over a physiological range tend to cause relaxation of vascular smooth muscle (38, 40). This relaxation and the resultant vasodilatation possibly play an important role in active and reactive hyperemia. Relaxation may be caused by hyperpolarization of the cell membrane due to its increased permeability to potassium (41). The moderate increase in extracellular potassium concentration may also impair propagation of excitation (42) and decrease the frequency of pacemaker firing (38). Greater increases in extracellular concentrations of potassium (over 15 mM) tend to cause contraction of smooth muscle probably due to cell membrane depolarization and increased permeability to calcium.

**ENERGY METABOLISM**

In vascular smooth muscle, metabolic processes supply energy to meet the demands of tissue function—in this case, contraction and relaxation. Also, effects of its own metabolism and of the metabolism of tissues in its environment, regulate the performance of the smooth muscle so that it is better able to meet the specialized requirements of the tissue in which it resides. In this way the metabolic processes serve both as an energy source and as a regulatory system for vascular smooth muscle function. The following three examples of the regulatory influence of metabolism illustrate the varied effects that metabolic processes can have on the performance of vascular smooth muscle.

When the metabolic requirements of a tissue are less than the requirement which is satisfied by the existing blood flow, vascular smooth muscle in that tissue contracts. The efficacy of this regulatory system is illustrated by Duling's observation (43) that the oxygen tension (Po2) of the tissue of a single layer of hamster cheek pouch remains constant as the Po2 of the solution bathing the tissue is increased from 11 to 47 mm Hg. This constancy of tissue Po2 is presumably maintained by a decrease in blood flow resulting from vasoconstriction (43). The vasoconstriction could result from either a decrease in the concentration of dilator metabolites resulting from the improved oxygen supply or a direct effect of increased Po2 on the vascular smooth muscle. The latter possibility seems unlikely from Duling's experiments, since Po2 measured on the surface of blood vessels supplying the cheek pouch actually decreased as the Po2 in the surrounding solution increased. Regardless of the mechanism, this regulatory system is important in effecting the increase in blood flow in both active and reactive hyperemia and in effecting the increase in resistance that results from an increase in perfusion pressure (flow autoregulation).

A second example of the regulatory function of metabolism on vascular smooth muscle is the closure of the ductus arteriosus that occurs when this structure is exposed to an elevated Po2. Superficially this function appears similar to the first example in which a direct relationship between Po2 and the degree of contraction of the muscle is observed. This relationship in ductus muscle is, however, quantitatively, and possibly also qualitatively, different. Fay (44) observed that smooth muscle of the isolated ductus arteriosus from the guinea pig fetus contracts in response to an increase in Po2 in a partial pressure-dependent fashion over a range of 0 to 140 mm Hg. Oxygen consumption by the tissue has the same Po2 dependence, and agents that block oxidative phosphorylation also block the contractile response to an elevated Po2. What is the mechanism by which the increased synthesis of adenosine triphosphate (ATP) via oxidative metabolism is translated into a contraction? The most obvious possibility, namely, that the ATP as an energy source for contraction is rate-limiting, apparently is not defensible, since in a
low Po2 environment (38 mm Hg) the muscle is still capable of developing three times the tension induced by Po2 alone when it is stimulated by acetylcholine. This increase in contractile response is not accompanied by a further increase in oxygen consumption by the tissue, suggesting that sufficient ATP is generated by a glycolytic pathway. The Po2-induced contraction of smooth muscle of the ductus arteriosus appears specifically dependent on the level of high-energy phosphate production by oxidative phosphorylation. An indirect mechanism that has been proposed (45) for the oxygen-induced contraction of the ductus arteriosus is that the increase in Po2 results in endogenous prostaglandin production, which causes the contraction. Possibly, this phenomenon, whatever its mechanism in the ductus arteriosus, also participates in the maintenance of nonneurogenic vascular tone in the adult.

A third example of a regulatory system mediated by the metabolism of vascular smooth muscle is seen in the pulmonary arterial tree where hypoxia may cause contraction of vascular smooth muscle and an increase in Po2 may cause its relaxation. Functionally, by decreasing blood flow to hypoxic alveoli, this regulatory system operates to help maintain a constant ventilation-perfusion ratio. Obviously, the mechanism responsible for an hypoxia-induced contraction must be qualitatively different from that which causes the increased contraction with elevated Po2, as described in the first two examples. Detar and his associates (46, 47) have observed that the phenomena of hypoxia-induced contraction and oxygen-induced relaxation develop much more readily in muscle from the pulmonary artery than in muscle from the aorta. Although the hypoxia-induced contraction is dependent to some extent on energy generated by anaerobic glycolysis, the response seems to reflect increased membrane permeability to activator calcium with hypoxia and decreased membrane permeability with elevated Po2. The interesting feature of the hypoxia-induced contraction and the oxygen-induced relaxation phenomena is that both are accentuated in the pulmonary artery by exposure of the tissue to hypoxia. If the hypoxia is adequately severe and if the exposure is prolonged (7 hours), these paradoxical changes develop in vascular smooth muscle from the aorta (47).

It is apparent from these three examples that the mechanisms relating vascular smooth muscle contraction or relaxation to metabolic events are numerous and complex. Each of the following metabolically dependent factors must be considered influential in the contractile state of this muscle: (1) concentration of ATP available for force development by the contractile proteins, (2) availability of ATP for sequestration of calcium and, hence, for relaxation of vascular smooth muscle, (3) the dilator effect of metabolic intermediates as they arise from either the vascular smooth muscle itself or from its parenchyma, (4) oxidative phosphorylation itself as a trigger for vascular smooth muscle contraction and myogenic tone, and (5) increases in plasma and mitochondrial membrane permeability under the influence of hypoxia, thereby altering the concentration of activator calcium or of metabolites that influence the contractile state of the vascular smooth muscle. Mechanistically, the details of the influence of metabolic processes on the contraction and relaxation of vascular smooth muscle have not been defined. Nevertheless, these processes are important in regulating the performance of this muscle.

Contraction and relaxation of vascular smooth muscle and the useful products of these processes depend on the functioning of the parts of the contractile machine that have been described. The function of each part is complex and alterable by physiological interventions. Energy metabolism has important regulatory functions. My ideas concerning the importance of nonelectrical activation in membrane excitation are radical and therefore should be regarded with suspicion. The evidence that I have presented, however, shifts the primary regulatory event for vascular smooth muscle contraction from membrane excitation to excitation-contraction coupling.

References


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