Stimulation of Vascular Smooth Muscle Sodium, Potassium–Adenosinetriphosphatase by Vasodilators

By Constantinos J. Limas and Jay N. Cohn

ABSTRACT

A ouabain-sensitive, Mg²⁺-dependent, Na⁺, K⁺-stimulated adenosinetriphosphatase (ATPase) isolated from canine mesenteric arteries was activated by the following vasodilators: hydralazine, diazoxide, PGE₁, PGE₂, PGA₁, and minoxidil. Epinephrine, norepinephrine, and isoproterenol also stimulated the ATPase, but PGF₂α was ineffective. Since these vasodilators activate the adenylyl cyclase of vascular smooth muscle, the effects of cyclic adenosine monophosphate (AMP) and theophylline on the Na⁺, K⁺-ATPase were studied; both substances caused a concentration-dependent increase in enzymatic activity. Propranolol blocked the catecholamine-induced stimulation of Na⁺, K⁺-ATPase, and polyphloretin phosphate antagonized the effects of the prostaglandins. It is concluded that vasodilatation in response to these substances is associated with the stimulation of the Na⁺, K⁺-ATPase of vascular smooth muscle probably mediated through cyclic AMP.

KEY WORDS

catecholamines
theophylline
mesenteric artery
prostaglandins
adenylate cyclase
diazoxide
minoxidil
propranolol
polyphloretin phosphate

Despite intensive investigation, the mechanisms that control contraction and relaxation of vascular smooth muscle remain uncertain. Considerable evidence, however, indicates that the availability of Ca²⁺ at contractile sites regulates both the initiation of contraction and the degree of relaxation of vascular smooth muscle (1, 2). Therefore, vascular contraction can be influenced by factors that affect transmembrane Ca²⁺ fluxes (3, 4).

Recent observations (5–8) have suggested that changes in intracellular cyclic 3', 5'-adenosine monophosphate (AMP) levels may be one of the mechanisms regulating smooth muscle function. Catecholamines stimulate adenylyl cyclase and, in proportion to this effect, decrease the contractile response of vascular smooth muscle; both adenylyl cyclase stimulation and vasodilatation are antagonized by β-receptor blocking agents. Relaxation of smooth muscle is also seen in the presence of phosphodiesterase inhibitors; these agents result in an increase in the intracellular levels of cyclic 3', 5'-AMP (8, 9). Angiotensin, in contrast, has been reported to decrease cyclic 3', 5'-AMP levels in vascular smooth muscle in proportion to its vasoconstrictor effects (7).

Since a number of vasodilators are known to stimulate the adenylyl cyclase of vascular smooth muscle (10), activation of this enzyme may be a common initial step in the action of these substances. The mechanism by which increased levels of cyclic 3', 5'-AMP lead to vasodilatation is not clear, although it probably involves a decrease in the concentration of Ca²⁺ in the vicinity of the contractile proteins of the smooth muscle cell. In the present study, we examined the effects of various vasodilators and of cyclic 3', 5'-AMP on the Na⁺, K⁺-adenosinetriphosphatase (ATPase) of vascular smooth muscle; this enzyme has been implicated in Ca²⁺ transport in other tissues (11).

Methods

Mesenteric arteries were obtained from dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) and subsequently killed by exsanguination. All of the steps in the isolation procedure were carried out in the cold (0°C). The tissue was rinsed free of blood with cold 0.25M sucrose (pH 7.2), cut into small pieces, and homogenized in a VirTis grinder (10% w/v in 0.25M sucrose) for 2 minutes. The whole homogenate (15-20 ml) was centrifuged at 1,200 × g for 20 minutes. The supernatant fluid was spun at 12,100 × g for 15 minutes, and the resultant sediment was discarded. The supernatant fluid was then centrifuged at 100,000 × g for 60 minutes to recover the microsomal fraction. For the isolation of mitochondria, the initial homogenate was centrifuged at 125 × g for 15 minutes, the supernatant fluid was decanted, and the cell debris was resuspended in four volumes of 0.25M
sucrose (pH 7.2). This suspension was spun at 285 g for 15 minutes; the two supernatant fractions were then combined and spun at 725 g for 10 minutes. The pellet was discarded, and the supernatant fluid was spun at 11,500 g for 20 minutes to obtain the mitochondrial fraction. An average of 2.54 mg of mitochondrial protein and 1.82 mg of microsomal protein was obtained from each gram of wet tissue. Characterization of the subcellular fractions was attempted by determining the activities of cytochrome c oxidase, 5'-nucleotidase, and D-nitrophenylphosphatase according to previously described methods (12-14). Protein was determined by the Biuret method (15).

The incubation medium for determination of Na+, K+, Mg2+-ATPase activity contained 5 mM MgCl2, 100 mM NaCl, 10 mM KCl, 50 mM histidine buffer (pH 7.6), 1 mM ethylenediaminetetraacetic acid (EDTA), and 100 mg of microsomal protein/ml in a total volume of 5 ml. The reaction was started by the addition of adenosine triphosphate (ATP) (final concentration 3 mM), and the mixture was incubated at 37°C for 30 minutes. The reaction was then stopped by the addition of 1 ml of cold 10% trichloroacetic acid; after centrifugation, inorganic P was determined in the supernatant fluid by the Lowry-Lopez method (16). Basal ATPase was defined as the Mg2+-independent ATP hydrolysis in the absence of NaCl and KCl where Mg2+-ATPase refers to the extra ATP splitting in the presence of Mg2+ and the absence of NaCl and KCl.

The effect of pH on the reaction rate determined over the range of 5.5 to 9.5. The concentration of ATP in the medium was varied between 10-4M and 10-6M. The effect of NaCl and KCl where Mg2+-ATPase refers to the extra ATP splitting in the presence of Mg2+ and the absence of NaCl and KCl.

Cyclic 3', 5'-AMP was assayed in the vascular wall by the method of Gilman (17), which is based on competition for protein binding of the nucleotide presumably to a cyclic AMP-dependent protein kinase in the presence of a protein kinase inhibitor. The binding reaction was conducted for 60 minutes at 0°C in a final volume of 150 ml of 50 mM sodium acetate buffer containing, in addition to the unknown amount of cyclic AMP, 14 ml of binding protein, 22.5 ml of protein kinase inhibitor, and 3H-cyclic AMP (0.06 pmole, specific activity 5-14 c/m mole). A standard curve was obtained by simultaneous determinations with unlabeled cyclic AMP in amounts ranging from 0 to 40 pmoles.

The following vasoactive substances were examined for their effects on Na+, K+-ATPase activity and cyclic 3', 5'-AMP levels: minoxidil (6-amino-1, 2-dihydro-1-hydroxy-2-imino-4-piperidinopirimidine), 1 hydroxalazine, diazoxide, prostaglandin E1 (PGE1), prostaglandin A1 (PGA1), catecholamines (norepinephrine, epinephrine, and isoproterenol), and theophylline. These vasoactive agents were added to the incubation medium for the ATPase assay as 0.1 ml of a solution containing 10-6-10-8 M test substance. In addition, the effect of cyclic 3', 5'-AMP on Na+, K+-ATPase was examined in the absence and the presence of CaCl2 (10-6-10-8 M). The incubation medium for the ATPase assay was identical to the one described earlier in this paper with the exception that EDTA was omitted. The influence of propranolol, a &-receptor blocking agent, on the catecholamine-induced changes in intracellular cyclic AMP and Na+, K+-ATPase was studied at a concentration of 20 &M (final concentration in the incubation medium). Finally, polyphloretin phosphate, a substance known to antagonize some of the effects of prostaglandins (18), was used to modify the effects of PGE1, PGE2, PGA2, and prostaglandin F2 (PGF2) on the Na+, K+-ATPase.

Results

The 100,000-g pellet designated the microsomal fraction contained only a small amount of mitochondrial as judged by the distribution of the marker enzymes (Table 1). Assays of Na+, K+-ATPase activity were carried out within 48 hours after preparation of the microsomal fractions despite the absence of deterioration of activity with cold storage for as long as 2 weeks. Basal ATPase activity was markedly stimulated by the simultaneous addition of Na+ and K+ to the incubation medium: 10.5 ± 1.7 moles P/mg protein hour-1 in the presence of 100 mM NaCl and 10 mM KCl compared with 2.7 ± 0.5 moles P/mg protein hour-1 when the medium contained all constituents except Na+ and K+ (P < 0.001). Addition of either 100 mM NaCl or 10 mM KCl alone did not change the ATPase activity (2.3 ± 0.5 moles P/mg protein hour-1 and 3.0 ± 0.6 moles P/mg protein hour-1, respectively, P = 0.5). Ouabain, in concentrations ranging from 10-6M to 10-4M, inhibited Na+, K+-ATPase (72% inhibition at 10-6M ouabain) but not Mg2+-ATPase (6.5 ± 1.0 moles P/mg protein hour-1 in the presence of 10-6M ouabain compared with 5.9 ± 1.2 moles P/mg protein hour-1 in its absence, P = 0.5).

The pH-activity curve showed a maximum around pH 7.4 for the Na+, K+-ATPase, but the Mg2+-ATPase was unchanged between 6.5 and 8.5. Substrate specificity differed for the Na+, K+-stimulated and the Mg2+-stimulated ATPase activities. In the case of the Na+, K+-dependent hydrolysis, not only ATP but also CTP could be utilized by the enzyme, although CTP was hydrolyzed to a much lesser degree: 2.7 ± 1.0 moles

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1Generously supplied by Dr. W. B. Martin of the Upjohn Company, Kalamazoo, Michigan.
2Generously supplied by Dr. J. E. Pike of the Upjohn Company, Kalamazoo, Michigan.
Distribution of Marker Enzymes in Different Fractions of Vascular Smooth Muscle Preparations

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Total homogenate</th>
<th>Mitochondrial fraction</th>
<th>Microsomal fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺-ATPase (µmoles P/mg protein hour⁻¹)</td>
<td>0.6 ± 0.04 (8)</td>
<td>1.0 ± 0.06 (8)</td>
<td>10.7 ± 1.4 (8)</td>
</tr>
<tr>
<td>5'-Nucleotidase (µmoles P/mg protein hour⁻¹)</td>
<td>1.9 ± 0.5 (6)</td>
<td>2.3 ± 1.0 (6)</td>
<td>18.9 ± 1.8 (6)</td>
</tr>
<tr>
<td>Cytochrome c oxidase (E₅₅₀/mg protein min⁻¹)</td>
<td>0.3 ± 0.07 (6)</td>
<td>0.1 ± 0.03 (6)</td>
<td>0.80 ± 0.07 (6)</td>
</tr>
<tr>
<td>p-Nitrophenylphosphatase (µmoles P/mg protein hour⁻¹)</td>
<td>(8)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

Number of experiments for each enzyme are given in parentheses. Results are expressed as means ± SE. E₅₅₀ = the decrease in absorbancy at 550 nm/min.

P/mg protein hour⁻¹ with CTP compared with 9.8 ± 1.4 µmoles P/mg hour⁻¹ with ATP (P < 0.01). Hydrolysis with ITP (2.1 ± 0.4 µmoles/mg hour⁻¹), GTP (1.8 ± 0.4 µmoles/mg hour⁻¹), UTP (1.9 ± 0.8 µmoles/mg hour⁻¹), and ADP (1.1 ± 0.3 µmoles/mg hour⁻¹) was quantitatively insignificant. In contrast, the basal Mg²⁺-dependent activity with either CTP, ITP, GTP, or UTP was almost the same order of magnitude as that with ATP. Na⁺, K⁺-ATPase activity increased linearly with ATP concentration from 10⁻⁸ to 10⁻⁴ M; Kₘ for ATP calculated from Lineweaver-Burk plots was 3.0 x 10⁻⁸ M.

Various types of inhibitors and reagents that modify ATPase activity were tested on the enzyme. As shown in Table 2, sodium azide, 2, 4-dinitrophenol, and histone had a negligible effect on the Na⁺, K⁺-ATPase. Oligomycin, p-chloromercuribenzoate, and N-ethylmaleimide specifically inhibited the Na⁺, K⁺-dependent activity of the enzyme, but they had little or no effect on the basal Mg²⁺-ATPase activity (Table 2).

Na⁺, K⁺-ATPase, but not Mg²⁺-ATPase, was stimulated by minoxidil, diazoxide, hydralazine, catecholamines, PGE₁, PGE₂, and PGA₂ but was unaffected by PGF₂α (Table 3); stimulation by agonists was dependent on their concentration in the reaction mixture. A representative dose-activity curve is shown in Figure 1 for minoxidil; the stimulatory effect was markedly attenuated by preincubation of the tissue with ouabain.

Basal cyclic 3', 5'-AMP concentration in the unstimulated mesenteric arteries was 1.2 ± 0.2 pmoles/mg protein; this concentration was significantly increased by all of the substances tested except PGF₂α (Table 3). In view of this finding, the effects of theophylline and cyclic 3', 5'-AMP on the Na⁺, K⁺-ATPase were tested. Theophylline at a concentration of 10⁻⁴ M resulted in a 60% stimulation of the enzyme (15.2 ± 1.4 µmoles P/mg protein hour⁻¹ in the presence of theophylline compared with 9.5 ± 1.0 µmoles P/mg protein hour⁻¹ in its absence). The effect of cyclic 3', 5'-AMP is shown in Figure 2; a concentration-dependent stimulation of Na⁺, K⁺-ATPase is evident. This stimulatory influence was attenuated by increasing the concentration of Ca²⁺ in the incubation medium. In the absence of cyclic 3', 5'-AMP, Ca²⁺ did not affect Na⁺, K⁺-ATPase activity.

Propranol (20 µM) attenuated the catechola-

### Table 1

<table>
<thead>
<tr>
<th>Additions</th>
<th>Na⁺, K⁺-ATPase (µmoles P/mg protein hour⁻¹)</th>
<th>P</th>
<th>Mg²⁺-ATPase (µmoles P/mg protein hour⁻¹)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>10.2 ± 1.6</td>
<td>0.01</td>
<td>6.7 ± 1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium azide (5 mM)</td>
<td>9.7 ± 0.7</td>
<td>NS</td>
<td>6.0 ± 1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2, 4-Dinitrophenol (10⁻⁴ M)</td>
<td>10.5 ± 1.5</td>
<td>NS</td>
<td>6.3 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Histone (10⁻⁴ M)</td>
<td>10.9 ± 1.3</td>
<td>NS</td>
<td>6.3 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Oligomycin (5 µg/ml)</td>
<td>3.6 ± 0.5</td>
<td>0.01</td>
<td>6.8 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate (10⁻⁴ M)</td>
<td>2.4 ± 0.7</td>
<td>0.001</td>
<td>6.5 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>N-Ethylmaleimide (10⁻⁴ M)</td>
<td>2.7 ± 0.7</td>
<td>0.001</td>
<td>6.6 ± 1.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results, expressed as means ± SE, were derived from six experiments for each substance. NS = not significant.

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TABLE 3

Effects of Vasodilators on the Na+, K+-ATPase Activity and Cyclic AMP Content of Vascular Smooth Muscle

<table>
<thead>
<tr>
<th>Additions (10^-8 M)</th>
<th>Na+, K+-ATPase (μmoles P/mg protein hour^-1)</th>
<th>Cyclic 3', 5'-AMP (pmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>10.6 ± 1.5</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>39 ± 1.7</td>
<td>2.34 ± 0.16</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>30 ± 1.4</td>
<td>2.16 ± 0.15</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>25 ± 0.8</td>
<td>2.0 ± 0.20</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>22 ± 0.9</td>
<td>1.9 ± 0.20</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>16 ± 1.1</td>
<td>1.8 ± 0.17</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>14 ± 1.0</td>
<td>1.6 ± 0.16</td>
</tr>
<tr>
<td>PGE₁</td>
<td>27 ± 1.3</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>PGE₂</td>
<td>25 ± 0.7</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>PGA₁</td>
<td>28 ± 1.0</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>PGF₁</td>
<td>9.8 ± 1.2</td>
<td>1.1 ± 0.12</td>
</tr>
</tbody>
</table>

Results, expressed as means ± se, are derived from six experiments for each substance.

mine-induced activation of Na+, K+-ATPase (Fig. 3) but did not have any direct inhibitory effect on the enzyme (Na+, K+-ATPase activity was 9.9 ± 1.3 μmoles P/mg protein hour^-1 in the presence of 20 μM propranolol and 10.2 ± 1.2 μmoles P/mg protein hour^-1 in its absence). A similar relationship could be demonstrated for the adenylate cyclase system and activation of Na+, K+-ATPase by PGE₁, PGE₂, and PGA₁. Polyphloretin phosphate blocked the prostaglandin-induced stimulation of Na+, K+-ATPase (Fig. 4). In contrast, it did not affect the enzyme directly: Na+, K+-ATPase activity was 9.0 ± 1.0 μmoles P/mg protein hour^-1 in the presence of 100 μg polyphloretin phosphate/ml incubation medium compared with 10.0 ± 1.7 μmoles P/mg protein hour^-1 in its absence (P = 0.5). Preincubation of the tissue with 10^-7 M
VASODILATORS AND Na⁺, K⁺-ATPase

Effect of 20 μM propranolol (hatched columns) on the catecholamine-induced (open columns) stimulation of Na⁺, K⁺-ATPase. The vertical axis represents the percent increase in enzymatic activity over control values. Vertical bars show ±SE.

FIGURE 3

Effects of prostaglandins and 100 μg polyphloretin phosphate (PPP)/ml on the Na⁺, K⁺-ATPase activity. Each point on the curves represents the average value from six experiments.

PGE₂ increased the intracellular cyclic AMP concentration from 1.1 ± 0.3 pmoles/mg protein to 1.7 ± 0.3 pmoles/mg protein (P < 0.005). Addition of 100 μg polyphloretin phosphate/ml incubation medium did not affect the PGE₂-induced increase in cyclic AMP (1.8 ± 0.5 pmoles/mg protein, indicating that polyphloretin phosphate blocks PGE₂-induced stimulation of Na⁺, K⁺-ATPase at a point distal to the generation of cyclic AMP. Addition of polyphloretin phosphate did not affect the activity of Mg²⁺-ATPase (6.5 ± 1.0 μmoles P/mg protein hour⁻¹ in the presence of 500 μg polyphloretin phosphate/ml compared with 6.7 ± 1.2 μmoles P/mg protein hour⁻¹ in its absence).

Discussion

Recent studies (1-4) have shown that free Ca²⁺ is the essential common requirement for all muscle contraction. The amount of tension developed by the contractile proteins is a direct function of the concentration of this ion to which they are exposed. There is a sparsity of data, however, on the mechanisms by which changes in extracellular and intracellular electrolytes alter the concentration of Ca²⁺ that plays this important role in the regulation of contraction (19). Several "pumps" have been described and implicated in the regulation of transmembrane Ca²⁺ fluxes. Baker and his associates (20) have demonstrated in the giant squid axon an active transmembrane electrolyte pump which couples Na⁺ efflux to Ca²⁺ influx. In the squid axon, Na⁺ extrusion from the cell is primarily dependent on the activity of the Na⁺, K⁺-stimulated, ouabain-sensitive ATPase which exchanges intracellular Na⁺ for extracellular K⁺. This enzyme has been isolated from a variety of tissues including cardiac (21) and vascular smooth muscle (22). The properties of the Na⁺, K⁺-ATPase from vascular smooth muscle reported in the present study, in terms of monovalent ion stimulation, substrate dependence, and effects of various inhibitors, closely resemble those previously described (21) for the cardiac enzyme, perhaps indicating a similarity of physiological roles. Another related system which may influence the contractile process is a Na⁺-Ca²⁺ exchange system in which the extrusion of Ca²⁺ from the cell is energized by the movement of Na⁺ into the cell down its electrochemical gradient. Similar pumps have been implicated in the regulation of Ca²⁺ fluxes in the vascular smooth muscle cell (3).

Tension changes as electrolyte concentrations vary and, in the presence of vasoactive substances, may be related to what would be expected from alterations of the Na⁺-Ca²⁺ pump. Because Na⁺ and Ca²⁺ compete for the influx system of this pump, Ca²⁺ influx should be exaggerated in a Na⁺-free environment; this phenomenon has, indeed, been observed (3). Further support for the role of this pump in the development of tension is given by the enhancement of contraction seen when the intracellular Na⁺ concentration is raised either by intracellular injection of Na⁺ into the myoplasm (23) or by ouabain-induced inhibition of the Na⁺-K⁺ pump.

The exact influence of vasodilator substances on Ca²⁺ movements has not been elucidated. It is clear, however, that relaxation of smooth muscle depends on mechanisms that decrease the intracellular Ca²⁺ concentration below the level which initiates contraction. Some of these mechanisms may involve a reversal or an inhibition of the previously described pumps which increase Ca²⁺ entry into the cell.
The results reported in the present paper allow the following formulation as one possible mechanism of action for some vasodilators. These substances stimulate the adenylate cyclase system of vascular smooth muscle and thereby increase the intracellular levels of cyclic AMP. This cyclic AMP, in turn, stimulates membrane Na\(^+\), K\(^+-\)ATPase, which results in a decrease in the intracellular levels of Na\(^+\) available for exchange with Ca\(^{2+}\) by the Na\(^+\)-Ca\(^{2+}\) pump. Consequently, the concentration of Ca\(^{2+}\) within the smooth muscle cell is decreased, and developed tension is decreased.

Support for the involvement of the Na\(^+\) pump in the action of vasodilators has come from recent experiments on the effects of prostaglandins. Kadar and Sunahara (24) have found that PGE\(_1\) inhibits spontaneous contractions of canine mesenteric arteries whereas PGF\(_2\alpha\) stimulates them. PGE\(_1\) has no effect on vessels pretreated with ouabain, but PGF\(_2\alpha\) still induces dose-dependent contractions. These observations are consistent with the proposition that the effects of PGE\(_1\) are dependent on an intact Na\(^+\), K\(^+-\)ATPase but that those of PGF\(_2\alpha\) are independent of the enzyme. They support the findings of the present study which implicate Na\(^+\), K\(^+-\)ATPase in the action of PGE\(_1\), but not PGF\(_2\alpha\).

Chen and his associates (25) have shown that in the dog gracilis muscle and forelimb ouabain suppresses or reverses hyperkalemic vasodilatation. They have suggested that deviations in plasma K\(^+\) concentrations elicit a change in the activity of the Na\(^+\), K\(^+-\)ATPase located at the membrane of the vascular smooth muscle cell. Stimulation of this enzyme in hyperkalemic results in hyperpolarization, which is known to lead to smooth muscle relaxation.

It is obvious that not all vasodilators can be expected to act by identical mechanisms, although, according to present concepts, they should all have a common final pathway—the reduction of intracellular Ca\(^{2+}\) concentration. It is possible for the adenylate cyclase activation step to be bypassed as is presumed to be the case with vasodilatation induced by K\(^+\), which seems to affect the Na\(^+\) pump directly. The Na\(^+\), K\(^+-\)ATPase activation step could also be bypassed by a vasodilator that inhibits the Na\(^+\)-Ca\(^{2+}\) pump directly. As yet, none of the presently available vasodilators has been shown to act through this mechanism. A third mechanism for modulating intracellular Ca\(^{2+}\) levels involves regulation of the ATP-dependent binding of Ca\(^{2+}\) to sarcoplasmic reticular vesicles (26). Angiotensin has been reported to decrease this Ca\(^{2+}\) binding, and cyclic AMP apparently increases it (27), thereby decreasing the amount of Ca\(^{2+}\) available to the contractile apparatus. Finally, vasodilators may act through a combination of these mechanisms with each mechanism having a different quantitative importance in enhancing relaxation of the vascular smooth muscle cell.

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