Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone

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Resistance arteries exist in a maintained contracted state from which they can dilate or constrict depending on need. In many cases, these arteries constrict to membrane depolarization and dilate to membrane hyperpolarization and Ca-channel blockers. We discuss recent information on the regulation of arterial smooth muscle voltage-dependent Ca channels by membrane potential and vasoconstrictors and on the regulation of membrane potential and K channels by vasodilators. We show that voltage-dependent Ca channels in the steady state can be open and very sensitive to membrane potential changes in a range that occurs in resistance arteries with tone. Many synthetic and endogenous vasodilators act, at least in part, through membrane hyperpolarization caused by opening K channels. We discuss evidence that these vasodilators act on a common target, the ATP-sensitive K (KATP) channel that is inhibited by sulfonylurea drugs. We propose the following hypotheses that presently explain these findings: 1) arterial smooth muscle tone is regulated by membrane potential primarily through the voltage dependence of Ca channels; 2) many vasoconstrictors act, in part, by opening voltage-dependent Ca channels through membrane depolarization and activation by second messengers; and 3) many vasodilators work, in part, through membrane hyperpolarization caused by KATP channel activation.

sulfonylurea drugs; vasodilation; vasoconstriction; smooth muscle; calcium channel blockers; membrane potential

ARTERIAL TONE, which underlies the maintenance of peripheral resistance in the circulation, is a major contributor to the control of blood pressure. The contractile force of arterial smooth muscle is regulated by the intracellular concentration of Ca2+ (62, 105). This is thought to occur by way of the dependence of myosin light chain phosphorylation on a kinase activated by the calcium-calmodulin complex. The phosphorylation leads to actin-myosin interaction and so to force development (57, 105, 113). Understanding the control of arterial tone thus requires knowledge of the regulation of intracellular Ca2+ as well as the contractile process. This has been the subject of much work, and a number of recent reviews have addressed aspects of the control mechanisms (28, 67, 75, 130). While transient contractions of vascular smooth muscle appear to involve release of Ca2+ from intracellular stores by inositol trisphosphate and possibly by Ca2+ itself (61, 113, 114), maintained contraction (i.e., tone) depends on the long-term balance between the entry of external Ca2+ (7, 22, 45, 59, 63, 96, 102) and its extrusion from the cell. Possible pathways for membrane Ca2+ entry include voltage-dependent Ca channels, hereafter also called Ca channels, receptor-operated channels (ROCs), and Na+-Ca2+ exchange. Ca2+ is extruded from smooth muscle by a plasma membrane adenosinetriphosphatase (ATPase) and Na+-Ca2+ exchange (22, 75, 111, 130). We will focus on Ca channels since they are targets of variety of vasodilators used in the treatment of high blood pressure (8, 71, 96, 130).

We propose the following three hypotheses, which we
consider to provide the most economical explanation of findings at present: 1) membrane potential controls arterial tone in large part through the voltage dependence of Ca channels; 2) many receptor-mediated agonists that induce vasconstriction act, in part, by increasing the open probability of Ca channels directly and through membrane depolarization; 3) many vasodilators act on arteries either directly by inhibiting the Ca channel or indirectly via the hyperpolarization caused by activation of ATP-sensitive K (K_{ATP}) channels. As well as providing evidence for these proposals, we will discuss alternative hypotheses and indicate gaps in current knowledge. We will only address Ca^{2+} extrusion and sarcoplasmic reticulum Ca^{2+} regulation (111, 113) as they relate to the aforementioned hypotheses.

We first discuss recent work on dihydropyridine-sensitive voltage-dependent Ca channels in arterial smooth muscle, concentrating on their membrane potential dependence and modulation by vasoconstrictors. We will consider these properties in relation to their possible role in the control of arterial tone. The final pathway for some vasodilations appears to be the effect of hyperpolarization, which is induced by the opening of K channels, on the voltage-dependent Ca channel. We subsequently discuss the evidence that a broad class of vasodilators have a common target in arterial smooth muscle, the K_{ATP} channel which is inhibited by sulfonylurea drugs such as glibenclamide.

VOLTAGE-DEPENDENT CALCIUM CHANNELS AND TONE

Membrane potential of arterial smooth muscle. A prerequisite to understanding the role of Ca channels in arteries is knowledge of the physiological range of membrane potentials exhibited by resistance arteries that have myogenic tone. Smooth muscle cells in arteries and arterioles, in vitro, have stable membrane potentials between -60 and -75 mV (67). Membrane potentials measured in vivo (90) are in the range of -40 to -55 mV (67). Resting potentials and the underlying resting permeability have been recently reviewed by Illing and Edwards (67). The resting membrane is mainly permeable to K^+. When cerebral (26, 58, 60), renal (60), and mesenteric resistance arteries (J. Brayden, personal communication) are subjected in vitro to physiological transmural pressures, they depolarize to between -40 and -55 mV and develop tone. Sympathetic nerve stimulation depolarizes arteries but rarely causes action potential generation (67). Furthermore, vasoconstrictors such as norepinephrine (NE) and serotonin appear to depolarize arterial smooth muscle (25, 54, 67, 88), and resistance arteries are likely to be under the tonic influence of such agents (67). For example, NE depolarized mesenteric arteries that were not subjected to transmural pressures, with the degree of depolarization depending on concentration [2-4 mV with 0.5 µM NE (91); <5 and 25 mV, with 10 µM NE in guinea pig (25) and rat (88), respectively]. Serotonin (10 µM) depolarized rabbit basilar arteries by 10 mV (54). The mechanism by which pressure and vasoconstrictors depolarize is unclear. In some cases, it might involve the activation of Ca-dependent chloride current and in others it may involve a sodium inward current (15, 29, 54, 67). The membrane potential of arterial smooth muscle cells in vivo (-40 to -55 mV) therefore falls in the same range in which the current through Ca channels is strongly voltage dependent. This Ca-channel current could thus have physiological relevance to tone maintenance. We consider this possibility in more detail below.

Membrane potential dependence of Ca channels. Ca channels, like other ion channels, switch between ion-conducting and -nonconducting conformation (open and closed, respectively). The proportion of time a Ca channel spends in the open or closed state is regulated by membrane potential. The steady-state fraction of time that a Ca channel is open [also called open-state probability or (P_{open})] increases exponentially with membrane depolarization from relatively hyperpolarized potentials such as -70 mV. This increase in P_{open} with membrane potential is limited by the promotion of a long-lived closed state called inactivated. Furthermore, the fraction of Ca channels that are functional (i.e., able to respond to membrane potential) may be subject to regulatory processes such as phosphorylation. Thus steady-state P_{open} can be expressed as the product of the open-state probability in the absence of inactivation (P_{act}), the probability of Ca channel not being in the inactivated state (1 - P_{inact}), and the probability that the channel is functional (P_{func}) (see Eq. A1 in the APPENDIX)

\[ P_{open} = (P_{act})(1 - P_{inact})(P_{func}) \]  \hspace{1cm} (1)

Voltage-dependent Ca channels in arteries exhibit inactivation during depolarizations to membrane potentials (V_m) more positive (depolarized beyond -40 mV) than those typically experienced by arterial smooth muscle (see above). Thus, provided that P_{func} is constant, P_{open} will reflect primarily activation or P_{act} and can be approximated by the following expression (see the APPENDIX for derivation)

\[ P_{open} \propto \frac{1}{1 + \exp\left(\frac{(V_{0.5} - V_m)}{k_e}\right)} \]  \hspace{1cm} (2)

where V_{0.5} is the membrane potential at which P_{open} is 0.5, and k_e is a steepness factor that gives an indication of the membrane potential sensitivity of Ca channel.

Furthermore, in the range of membrane potentials exhibited by resistance arteries that have myogenic tone (-55 to -40 mV), maintained Ca currents and Ca channel P_{open} (see below) are a small fraction of their values at more positive potentials, suggesting that Ca channels in resistance arteries normally function at low levels of activation. Under this condition, steady-state P_{open} is the most sensitive to membrane potential and Eq. 2 can be further simplified to the following approximation.

\[ P_{open} \propto \exp\left(\frac{V_m}{k_e}\right) \]  \hspace{1cm} (3)

Thus, for P_{open} < 0.12 of the maximal P_{open}, P_{open} will increase e-fold for a depolarization of k_e millivolts, with no more than 13% deviation from Eq. 1. Relationships between steady-state P_{open} and membrane potentials between -60 and -30 mV using Eqs. 1-3 are illustrated in Fig. 1D (using -25 mV as the midpoint of both the
steady-state activation and inactivation curves). The relationships are natural log transformed to illustrate the exponential relationship between \( P_{\text{open}} \) and \( V_m \) in this range. Under these conditions, \( P_{\text{open}} \) between \(-60\) and \(-40\) mV is well described by Eq. 3 and is essentially an exponential function of membrane potential. Hyperpolarizing agents (e.g., hyperpolarizing vasodilators, see below) would be clearly an effective way to lower \( P_{\text{open}} \), since the relationship between \( P_{\text{open}} \) and \( V_m \) becomes steeper as \( V_m \) hyperpolarizes. Under this condition, a
membrane hyperpolarization of 2 mV could decrease $P_{\text{open}}$ and thus Ca$^{2+}$ entry by ~30%. Any agent that increases inactivation would make $P_{\text{open}}$ less sensitive to changes in $V_m$. Membrane depolarization beyond ~40 mV would not be the most effective method to increase $P_{\text{open}}$, since inactivation and decrease in driving force would tend to offset increases in activation. However, agents that shift the activation curve to more negative potentials (e.g., BAY K 8644) would increase $P_{\text{open}}$.

It should be stressed that $P_{\text{open}}$ is a continuous function of membrane potential, with no threshold (65); in other words, a Ca channel always has a finite $P_{\text{open}}$. Apparent “thresholds” for a whole cell Ca currents (or depolarization-induced force development) are simply detection thresholds; Ca channels will certainly be open negative to these potentials, albeit with low $P_{\text{open}}$.

**Measurement of Ca channel steady-state behavior.** The predominant voltage-dependent Ca channel in arterial smooth muscle is inhibited by the dihydropyridine Ca-channel blockers and inactivates slowly and incompletely during prolonged depolarizations. It has been referred to as an “L-type” Ca channel (128). A dihydropyridine-insensitive, rapidly inactivating (called “T”) type of Ca-channel current has been reported in some (11, 17) but not all (1, 93) types of arterial smooth muscle. We will concentrate on measurements of the former (L) type of channel, since maintained arterial tone is strongly inhibited by dihydropyridines and since transient Ca$^{2+}$ fluxes are likely to play a minimal role in the steady state.

Experimentally, the parameters, $k_e$ and $V_{0.5}$, for approximating the voltage dependence of Ca-channel activity can be determined by estimating $P_{\text{open}}$ (or some parameter directly proportional to it, for example, current) over a range of $V_m$. Ca-channel activation has been investigated both at the whole cell and single channel level. Whole cell recording allows measurement of currents in physiological extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]o) and also measures currents averaged from many channels. However, the nonlinear instantaneous current-voltage relation, uncertainty about the reversal potential for currents through Ca channels, and currents attributable to other types of channels make it difficult to measure accurately the Ca conductance and thus an activation curve from macroscopic currents. Because the number of channels (as well as the current through a single channel) participating in the whole current responses is generally unknown, $P_{\text{open}}$ usually cannot be determined. It is also difficult to detect very small Ca currents, for example, at negative potentials, because these may be obscured by leakage or other outward currents. A further problem with conventional patch whole cell recording is disruption of the native cytoplasmic composition by diffusion from the pipette. This last problem may be overcome by using microelectrodes (68) or by the nystatin permeabilization method (69).

Single-channel recording, on the other hand, allows an absolute measure of $P_{\text{open}}$ and so of the activation curve. It can also measure very low $P_{\text{open}}$ values and can measure directly the Ca-channel activity at steady membrane potentials. Finally, it allows measurement on intact cells. Disadvantages with this method are the need to average many recordings to measure a time course of $P_{\text{open}}$. Also, in the cell-attached patch, the cell’s resting potential must be eliminated or estimated independently.

Because single Ca-channel currents are small in physiological [Ca$^{2+}$], and their openings are brief at negative potentials, most measurements of single Ca channels have been made using high concentrations of Ba$^{2+}$ as a charge carrier to maximize the single channel current. In addition, BAY K 8644 or its active isomer, BAY R 5417, has been used to increase single-channel open time. High concentrations of Ba$^{2+}$, and the use of BAY K 8644, although convenient, also alter the properties of the channels under study. These problems can be overcome, however, with sufficiently high-resolution recordings. For example, Fig. 1A shows recordings of single Ca channels at steady $V_m$ of ~50 mV with 10 mM Ca$^{2+}$ as the charge carrier and no BAY K 8644 from an on-cell patch of single smooth muscle cell from the rabbit basilar artery. The complete characterization of Ca channels can thus be accomplished using a combination of techniques.

**Membrane potential dependence of Ca channels.** Macroscopic Ca currents can be detected under voltage clamp at potentials between ~60 and ~45 mV in physiological [Ca$^{2+}$], (1, 68). Single-microelectrode voltage-clamp recordings in cerebral arterioles (68) and whole cell patch-clamp measurements in rabbit car artery (1) demonstrate that modest depolarizations (to potentials negative to ~40 mV) activate a maintained dihydropyridine-sensitive inward Ca current that does not inactivate significantly (with physiological Ca$^{2+}$ as the charge carrier). These studies also indicate a very steep relationship between membrane potential and maintained Ca current for membrane potentials negative to ~40 mV, with this current increasing three to fourfold between ~50 and ~40 mV.

To date, measurements of the voltage dependence of $P_{\text{open}}$ of single Ca channels in arteries are limited to the mesenteric artery, cerebral arteries, and coronary arteries (53, 91, 102, 103, 136). The voltage dependence of Ca channels can be illustrated over a wide voltage range by using Ba$^{2+}$ as a charge carrier and with BAY R 5417 (Fig. 1, B and C). In this experiment, $P_{\text{open}}$, was 0.006 at ~60 mV and increased to 0.041 at ~40 mV. The relationship between $V_m$ and $P_{\text{open}}$ over this $V_m$ range can be described by Eq. 3 using a steepness factor of 8.5 mV, i.e., $P_{\text{open}}$ changes e-fold per 8.5 mV for membrane potentials negative to ~40 mV (Fig. 1C). The relationship between $V_m$ and $P_{\text{open}}$ over a wider voltage range can be described by Eq. 2, using $V_{0.5} = -23.5$ mV, $P_{\text{funct}} = 0.36$, and $k_e = 8.5$ mV (Fig. 1C, inset). In general, the midpoints of the activation curve ($V_{0.5}$) for Ca channels in smooth muscle cells from rabbit mesenteric artery fall between ~10 and ~25 mV (92) and those from rabbit basilar artery between 0 and +10 mV (Worley, Standen, and Nelson, unpublished observations), respectively, with Ba$^{2+}$ as the charge carrier and in the dihydropyridine agonist BAY R 5417.

BAY K 8644 shifts the activation curve ~10 mV in the negative direction, and Ba$^{2+}$ shifts the activation curve ~20 mV in the positive direction (1, 53, 108). This
suggests that the midpoint of the activation curve in mesenteric arteries under more physiological conditions would be between −20 and −35 mV. Exact determination of this midpoint will of course require studies of the activation of single Ca channels in physiological [Ca$^{2+}$]o and temperature and in the absence of dihydropyridine agonists. It is, nevertheless, likely that over the physiological range of resting potentials, −55 to −40 mV, Ca channels are operating well below half-activation.

The preceding paragraphs have demonstrated that Ca channels will have a finite, albeit low, Popen over the physiological range of Vm (−55 to −40 mV). For example, in the experiment of Fig. 1A, steady-state Popen at −50 mV was −0.003. It is relevant to ask whether Ca channels with such low Popen are sufficient to play a major role in the steady balance of Ca$^{2+}$ influx and exit from the cell. Ca$^{2+}$ influx through even a small number of Ca channels is sufficient to lead to significant changes in intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]i), because of the small volume of arterial smooth muscle cells (~1 pl (1)). The single-channel Ca current at −50 mV in 1.8 mM [Ca$^{2+}$]o can be estimated to be ~0.07 pA from the single-channel current of ~0.38 pA in 10 mM [Ca$^{2+}$]o and the dissociation constant (Kd) for Ca$^{2+}$ of 13.9 mM (64) at 22°C. A unitary current of 0.07 pA corresponds to a Ca$^{2+}$ influx rate of ~200,000 ions/s through a single open Ca channel. Thus just one open channel in a single cell would deliver Ca$^{2+}$ at a rate sufficient to raise [Ca$^{2+}$]i, 0.3 μM/s at 22°C and by at least 1 μM/s at 37°C (32) in the absence of buffering and extrusion. Because each smooth muscle cell has >1,000 such Ca channels (135), the cell would experience substantial Ca$^{2+}$ influx even at Popen as low as 0.001. The final change in steady-state [Ca$^{2+}$], resulting from Ca$^{2+}$ entry will ultimately depend on the way in which Ca$^{2+}$ is extruded from the cell (12). Thus Ca$^{2+}$ entry through Ca channels over the physiological range of membrane potentials could supply smooth muscle cells with sufficient Ca$^{2+}$ to maintain steady contraction.

Ca-channel activation by vasoconstrictors. Regulation of function through the modulation of voltage-dependent Ca channels by neurotransmitters and neuropeptides appears to be a widespread phenomenon (9, 98, 128). Only recently have direct demonstrations of modulation of arterial smooth muscle Ca channels been provided. However, in arterial smooth muscle, it has been proposed that vasoconstrictors increase Ca$^{2+}$ entry by opening Ca-permeable voltage-independent channels (called ROCS). The nature of the Ca$^{2+}$ entry pathway activated by vasoconstrictors would have profound implications on how arterial smooth muscle responds to changes in Vm. For example, membrane hyperpolarization would increase Ca$^{2+}$ entry through ROCS by increasing the driving force for Ca$^{2+}$ movement, whereas it would decrease Ca$^{2+}$ influx through voltage-dependent Ca channels by reducing their Popen.

Vasoconstrictors such as NE appear to act through multiple synergistic pathways to increase force. 1) NE can increase [Ca$^{2+}$], transiently by causing release from the sarcoplasmic reticulum (61, 114). 2) Some vasoconstrictors may shift the relationship between [Ca$^{2+}$]o and force such that there is a higher level of steady-state force for a given concentration of [Ca$^{2+}$]o (94, 115). Nevertheless, steady-state force would still depend on [Ca$^{2+}$]. 3) Vasoconstrictors could decrease Ca$^{2+}$ extrusion. 4) Vasoconstrictors also cause a prominent increase in Ca$^{2+}$ entry by opening voltage-dependent Ca channels. Ca$^{2+}$ entry is also increased in the absence of significant depolarization (24, 28, 113, 116, 129, 130). Below, we discuss evidence that vasoconstrictors can increase Popen of voltage-dependent Ca channels even in the absence of Vm changes.

NE increased Popen of single Ca channels recorded in patches on single cells isolated from rabbit mesenteric artery (92) and also increased whole cell Ca channel currents in voltage-clamped single smooth muscle cells from rabbit ear artery cells (19). NE has also been shown to increase and decrease Ca-channel currents in cultured cells from rat portal vein (97). The action of serotonin in increasing single Ca channel Popen in cells from basilar artery (136) is shown in Fig. 2. NE (10 μM) increased Popen of Ca channels in mesenteric artery by up to four-fold (92), whereas serotonin was a much more potent activator of Ca channels in basilar artery; 100 nM serotonin increased Popen as much as 86-fold. On cell single Ca-channel experiments are made in solutions that null the cell's membrane potential and minimize membrane

FIG. 2. Serotonin (100 nM) activation of single Ca channels. Currents were recorded from a patch on a cell from a rabbit cerebral (basilar) artery. The patch was stepped to 0 mV from a holding potential of −75 mV. Solid line indicates the closed level, and dashed line indicates the open level, filtered at 800 Hz. Bath and pipette solutions are as in Fig. 1B.
potential changes. Furthermore, because the amplitude of the single-channel current, which would decrease with membrane depolarization, was unchanged, it is clear that these transmitters are not activating Ca channels by causing depolarization. In whole cell experiments (19), NE activation of currents through voltage-dependent Ca channels was seen without change in the (voltage-clamped) \( V_m \).

One possibility that is suggested by the activation curves for single channels and for contractions (92) is that NE and serotonin shift the activation curve for Ca channels to the left so that \( P_{\text{open}} \) is higher at any given \( V_m \). A slight shift in the activation curve could cause a substantial increase in \( Ca^{2+} \) entry. NE could also increase the probability of a channel being functional, \( P_{\text{funct}} \). These actions of NE and serotonin on Ca channels may represent a general mechanism contributing to vasoconstriction. For example, angiotensin II (unpublished results and Ref. 20) and the endothelium-derived vasoconstrictor peptide endothelin also open voltage-dependent Ca channels (56, 112, 138). Because the vasoconstricting neurotransmitters described above activate Ca channels, it seems possible that some vasodilators may act by reducing \( P_{\text{open}} \) of these channels, i.e., by shifting the activation curve in the positive direction or by decreasing \( P_{\text{funct}} \) (cf. Ref. 11). This will of course depend on Ca channel \( P_{\text{open}} \) being sufficiently high at physiological membrane potentials for downmodulation to reduce tone, as we discuss below. Constrictor activation of Ca channels may be a property of smooth muscle generally, since acetylcholine has been shown to activate Ca currents in stomach smooth muscle (35).

In the single-channel experiments described above, Ca currents were recorded from a cell-attached patch while NE or serotonin were applied only to the solution bathing the extrapatch membrane. This suggests that an intracellular second messenger may mediate the modulation of Ca channels by these vasoconstrictors. In arterial smooth muscle, \( \alpha \)-receptor stimulation by NE activates phospholipase C, which causes the formation of inositol triphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol diphosphate (61). IP3 can cause release of \( Ca^{2+} \) from sarcoplasmic reticulum, which appears to be responsible for transient contractions of smooth muscle (61, 114). Agonist-induced release of sarcoplasmic reticulum \( Ca^{2+} \) is prominent in large conduit arteries but smaller when the membrane is depolarized by high K+ (8, 45, 130). DAG promotes the formation of the \( Ca^{2+} \)-sensitive form of protein kinase C. It is possible that a protein kinase C-mediated phosphorylation increases \( P_{\text{open}} \) of arterial smooth muscle Ca channels (51). Protein kinase C has been shown to cause the phosphorylation of the \( \alpha_1 \)- and \( \beta \)-subunits of the purified dihydropyridine binding protein of skeletal muscle (30). Phosphorylation of the Ca channels of cardiac and skeletal muscle has been shown to increase \( P_{\text{open}} \) (73, 106). It is also possible that \( \alpha \)-receptor stimulation opens Ca channels through a G protein pathway in arterial smooth muscle (27). An important area of future work in arterial smooth muscle will be the elucidation of the second messenger systems involved in the modulation of Ca channels by neurotransmitters and peptides.

The demonstration that voltage-sensitive Ca channels can be activated by catecholamines necessitates a reexamination of the possible nature of a catecholamine-activated \( Ca^{2+} \) influx pathway, ROCs (24, 28, 86, 116, 129, 130). It has been proposed that ROCs are voltage-independent \( Ca \)-permeable channels distinct from voltage-dependent Ca channels (86). Such catecholamine-activated ROCs have not been identified as single channels, although nonselective divalent cation-permeable channels activated by external ATP have been found in rabbit ear artery (18) and divalent cation-permeable channels from thrombin-activated platelets have also been reported (139). Furthermore, the structure of adrenergic receptors is not consistent with them forming ion channels directly (63, 121).

The main evidence in favor of the existence of ROCs operated by \( \alpha \)-receptors, as distinct from voltage-dependent Ca channels, comes from experiments measuring tension or \( { }^{45}Ca \) influx (75). First, NE can increase \( { }^{45}Ca \) influx and force without substantial depolarization. Second, NE can elicit extra tension and influx in aortic smooth muscle already stimulated by high-K+ depolarization. Third, the \( Ca^{2+} \) uptake in response to simultaneous application of NE and high K+ approximates the sum of that produced by either agent alone. Fourth, \( Ca^{2+} \) influx in response to NE in aorta is less sensitive to organic Ca-channel blockers than is the influx induced by depolarization.

These observations may now also be reconciled with the known properties of voltage-dependent Ca channels. First, NE as well as serotonin, angiotensin II, and endothelin have been shown to increase voltage-dependent Ca channels without a change in membrane potential.

Second, high K+ would not lead to complete activation of voltage-dependent Ca channels so that an additional contraction on the addition of NE would be expected. Even at very depolarized levels, the steady-state \( P_{\text{open}} \) is well below one so that NE could open Ca channels by decreasing inactivation or by increasing \( P_{\text{funct}} \).

Third, for similar reasons, a greater \( Ca^{2+} \) influx would be expected with NE and high K+ together than when either is presented alone. In fact, the approximate additivity of the \( Ca^{2+} \) influxes seen with high K+ and NE (75, 86) is at variance with the idea of a separate \( Ca^{2+} \) permeable ROC, since the \( Ca^{2+} \) influx induced by NE through such a channel would be expected to be much smaller when the membrane is depolarized by high K+ than at the resting potential, because of the reduced driving force on Ca2+.

Fourth, the higher sensitivity of depolarization-induced \( Ca^{2+} \) influx to organic blockers may be a consequence of the voltage-dependent nature of the blocking action of these agents (87, 93), with membrane depolarization promoting inhibition. In other words, a lower inhibition constant will be measured at a depolarized potential than at a more negative one, even though the target channel is the same in each case. Furthermore, NE activation of voltage-dependent Ca channels in arterial smooth muscle may reduce their affinity for Ca-
channel blockers. For example, voltage-dependent Ca channels stimulated by epinephrine in cardiac muscle become relatively insensitive to inhibition by D 600 (84). NE-induced decrease in Ca-channel blocker inhibition of Ca channels in aorta may explain the observation that cromakalim (a hyperpolarizing drug) but not isradipine (a dihydropyridine Ca-channel antagonist) reduced NE-activated maintained contractions of aorta (39, 41). Also, it has been shown the G proteins can modulate Ca-channel antagonist and agonist action (98). Another important area of future work will be the examination of the effects of agonist activation of voltage-dependent Ca channels on their pharmacology.

The above arguments do not, of course, exclude the possibility that NE activated voltage-insensitive Ca-permeable channels exist in some types of smooth muscle. It is possible that future single-channel experiments will convincingly demonstrate ROCs that are distinct from the voltage-dependent Ca channel. However, because presently there is no direct evidence for the existence of a separate class of NE-activated voltage-insensitive Ca-permeable channels and because voltage-sensitive Ca channels are activated by NE, it may be that NE receptor-operated channels and voltage-dependent Ca channels are in fact identical.

**Proposed role for voltage-sensitive Ca channels in the regulation of tone.** We propose that voltage-dependent Ca channels play an important role in controlling steady-state Ca entry, and thus tone of smooth muscle in resistance-sized arteries. As we have seen, dihydropyridine-sensitive Ca channels are active in the same range of potentials as is observed in pressurized arteries (1, 67, 68 and Fig. 1A). Because the total Ca flux through these channels is strongly dependent on Vm, alterations in Vm would play a preeminent role in controlling steady tone. A consequence of such a mechanism would be that anything causing even slight hyperpolarization of these smooth muscle cells could decrease tone, while slight depolarization could increase it. Furthermore, vasoconstricting neurotransmitters or intracellular second messengers may alter the responses of the voltage-dependent Ca channels at a given Vm, thus modulating Ca entry and tone without requiring a change in Vm. We discuss below the justification for and the plausibility of these hypotheses.

The hypothesis that voltage-sensitive Ca channels are involved in the maintenance of tone in resistance vessels is supported by the following evidence. First, blockers of voltage-sensitive Ca channels are known to decrease arterial pressure in vivo by causing vasodilation, both in normal and hypertensive states (71). A number of anti-hypertensive and antiangiinal drugs fall into this group, for example, verapamil, diltiazem, and nifedipine.

Tone of resistance-sized cerebral and systemic arteries in vitro is dependent on external Ca and can be abolished by Ca-channel blockers (45, 71, 130). For example, resistance-sized cerebral, mesenteric, and renal arteries subjected to physiological transmural pressures exhibit spontaneous (myogenic) tone that is dependent on external Ca (58, 60) and completely abolished by Ca-channel inhibitors (59, 63, 96, 102) and membrane hyperpolarization (26).

These observations support our proposition that tone should depend on extracellular Ca, and they show that Ca entry must be through a pathway that is sensitive to known Ca-channel blocking agents. Because Ca channels have been demonstrated in these tissues, they provide sufficient explanation of these observations.

Second, voltage sensitivity for the voltage-dependent changes in tone is similar to that of the Ca channel (91), with both Popen and tension changing ~e-fold for 6–8 mV over the physiological range of membrane potentials. The relationship between membrane Vm and 46Ca entry is also steep (75). This would predict that steady-state [Ca], should depend on Vm increasing with membrane depolarization, with its exact level depending on the steady-state balance between Ca entry and extrusion. These predictions should be now testable in voltage-clamped single smooth muscle cells loaded with fura-2 or other Ca indicators, and it will be important to measure the effects of small changes in Vm and of Ca-channel blockers on [Ca], using these techniques (12, 137).

Third, factors causing depolarization increase tone. The application of physiological pressures causes cerebral arterial smooth muscle cells to depolarize from −63 mV to between −50 and −40 mV (26). Although the mechanism and the underlying ionic basis of the pressure-induced depolarization is not known (and will be an important area of future investigation), it is clear that such depolarization is associated with an increase in tone. For example, increasing the transmural pressure to a physiological level (80 mmHg) caused a middle cerebral artery from rabbit to constrict from a diameter of 390 to 270 µm (Fig. 3A). As with other types of tone described above, nisoldipine, a dihydropyridine blocker of arterial smooth muscle Ca channels (74, 93), relaxed this pressurized artery (Fig. 3A), suggesting that the entire tone depended on Ca entry through Ca channels. A similar relation holds between tone and membrane depolarization induced by other treatments. In particular, depolarization of the membrane with high K+ is well known for its ability to produce tone. For example, the effect of Vm on steady-state force is illustrated in Fig. 3B.

In all cases, therefore, the relationship between Vm and tone is steep in the potential range between −65 and −40 mV. The simplest explanation for this voltage dependence is that the primary entry pathway is the Ca channel.

Fourth, factors causing hyperpolarization decrease tone. Agents that cause membrane hyperpolarization are potent vasodilators in vitro and in vivo (39, 99, 100, 132). Although the quantitative relationship between the degree of hyperpolarization and the amount of reduction in tone has yet to be determined, tone changes have been demonstrated in arteries that have undergone only slight hyperpolarization (26). As with depolarization, this relationship can best be explained by membrane hyperpolarization decreasing Ca entry (34, 92) by closing Ca channels.

Alternate mechanisms for Vm regulation of tone in-
channel blockers. For example, voltage-dependent Ca channels stimulated by epinephrine in cardiac muscle become relatively insensitive to inhibition by D 600 (84). NE-induced decrease in Ca-channel blocker inhibition of Ca channels in aorta may explain the observation that cromakalim (a hyperpolarizing drug) but not isradipine (a dihydropyridine Ca-channel antagonist) reduced NE-activated maintained contractions of aorta (39, 41). Also, it has been shown the G proteins can modulate Ca-channel antagonist and agonist action (98). Another important area of future work will be the examination of the effects of agonist activation of voltage-dependent Ca channels on their pharmacology.

The above arguments do not, of course, exclude the possibility that NE activated voltage-insensitive Ca-permeable channels exist in some types of smooth muscle. It is possible that future single-channel experiments will convincingly demonstrate ROCs that are distinct from the voltage-dependent Ca channel. However, because presently there is no direct evidence for the existence of a separate class of NE-activated voltage-insensitive Ca-permeable channels and because voltage-sensitive Ca channels are activated by NE, it may be that NE receptor-operated channels and voltage-dependent Ca channels are in fact identical.

Proposed role for voltage-sensitive Ca channels in the regulation of tone. We propose that voltage-dependent Ca channels play an important role in controlling steady-state Ca entry, and thus tone of smooth muscle in resistance-sized arteries. As we have seen, dihydropyridine-sensitive Ca channels are active in the same range of potentials as is observed in pressurized arteries (1, 67, 68 and Fig. 1A). Because the total Ca flux through these channels is strongly dependent on Vm, alterations in Vm would play a preeminent role in controlling steady-state Ca entry and thus tone. A consequence of such a mechanism would be that anything causing even slight hyperpolarization of these smooth muscle cells could decrease tone, while slight depolarization could increase it. Furthermore, voltage-sensitive Ca channels at a given Vm, thus modulating Ca entry and tone without requiring a change in Vm. We discuss below the justification for and the plausibility of these hypotheses.

The hypothesis that voltage-sensitive Ca channels are involved in the maintenance of tone in resistance vessels is supported by the following evidence. First, blockers of voltage-sensitive Ca channels are known to decrease arterial pressure in vivo by causing vasodilation, both in normal and hypertensive states (71). A number of antihypertensive and antianginal drugs fall into this group, for example, verapamil, diltiazem, and nifedipine.

Tone of resistance-sized cerebral and systemic arteries in vitro is dependent on external Ca(2+) and can be abolished by Ca-channel blockers (45, 71, 130). For example, resistance-sized cerebral, mesenteric, and renal arteries subjected to physiological transmural pressures exhibit spontaneous (myogenic) tone that is dependent on external Ca(2+) (58, 60) and completely abolished by Ca-channel inhibitors (59, 63, 96, 102) and membrane hyperpolarization (26).

These observations support our proposition that tone should depend on extracellular Ca(2+), and they show that Ca(2+) entry must be through a pathway that is sensitive to known Ca-channel blocking agents. Because Ca channels have been demonstrated in these tissues, they provide sufficient explanation of these observations.

Second, voltage sensitivity for the voltage-dependent changes in tone is similar to that of the Ca channel (91), with both Popen and tension changing -e-fold for 6-8 mV over the physiological range of membrane potentials. The relationship between membrane Vm and Ca(2+) entry is also steep (75). This would predict that steady-state [Ca(2+)] should depend on Vm, increasing with membrane depolarization, with its exact level depending on the steady-state balance between Ca(2+) entry and extrusion. These predictions should be now testable in voltage-clamped single smooth muscle cells loaded with fura-2 or other Ca(2+) indicators, and it will be important to measure the effects of small changes in Vm and of Ca-channel blockers on [Ca(2+)2], using these techniques (12, 137).

Third, factors causing depolarization increase tone. The application of physiological pressures causes cerebral arterial smooth muscle cells to depolarize from -63 mV to between -50 and -40 mV (26). Although the mechanism and the underlying ionic basis of the pressure-induced depolarization is not known (and will be an important area of future investigation), it is clear that such depolarization is associated with an increase in tone. For example, increasing the transmural pressure to a physiological level (80 mmHg) caused a middle cerebral artery from rabbit to constrict from a diameter of 390 to 270 µm (Fig. 3A). As with other types of tone described above, nisoldipine, a dihydropyridine blocker of arterial smooth muscle Ca channels (74, 93), relaxed this pressurized artery (Fig. 3A), suggesting that the entire tone depended on Ca(2+) entry through Ca channels. A similar relation holds between tone and membrane depolarization induced by other treatments. In particular, depolarization of the membrane with high K+ is well known for its ability to produce tone. For example, the effect of Vm on steady-state force is illustrated in Fig. 3B.

In all cases, therefore, the relationship between Vm and tone is steep in the potential range between -65 and -40 mV. The simplest explanation for this voltage dependence is that the primary entry pathway is the Ca channel.

Fourth, factors causing hyperpolarization decrease tone. Agents that cause membrane hyperpolarization are potent vasodilators in vitro and in vivo (39, 99, 100, 132). Although the quantitative relationship between the degree of hyperpolarization and the amount of reduction in tone has yet to be determined, tone changes have been demonstrated in arteries that have undergone only slight hyperpolarization (26). As with depolarization, this relationship can best be explained by membrane hyperpolarization decreasing Ca(2+) entry (94, 92) by closing Ca channels.

Alternate mechanisms for Vm regulation of tone in-
Cl0 BRIEF REVIEW

A 400q

Nisoldipine (100nM)

FIG 3. A: nisoldipine dilation of a pressurized middle cerebral artery from rabbit. Transmural pressure was 80 mmHg. Horizontal line marks initial diameter of the relaxed artery before pressurization (J. E. Brayden, unpublished experiment). B: membrane depolarization increased force of rabbit mesenteric arteries. Artery was depolarized by elevating external potassium from 5 (-61.2 mV) to 20 mM (-41.7 mV), and force increased ~10-fold (see also Ref. 92).

Membrane depolarization would decrease, not increase, Ca²⁺ entry through ROCs by reducing the Ca²⁺ electrochemical gradient. Conversely, membrane hyperpolarization would increase Ca²⁺ entry through such a channel and cause vasodilation. This is contrary to what is observed. Therefore, it seems unlikely that such a channel is responsible for the voltage dependence of tone.

Na⁺-Ca²⁺ exchange is dependent on Vm, with membrane depolarization decreasing net Ca²⁺ extrusion by the transporter (23, 76, 81). This would tend to increase [Ca²⁺], and thus force. However, Na⁺-Ca²⁺ exchange appears to be far less sensitive to Vm than Ca channels (over the physiological range). For example, >30-mV depolarization is required to change the transporter rate e-fold (23, 76, 81), whereas 6–8 mV increases Popen e-fold for the Ca channels. Nevertheless, if Na⁺-Ca²⁺ exchange is regulating arterial smooth muscle free Ca²⁺, then it would be expected to contribute in a small way to the overall voltage dependence of [Ca²⁺]. Examination of the voltage dependence of [Ca²⁺] and tone in the absence of Na⁺-Ca²⁺ exchange (e.g., in Na⁺-free solutions) should provide information about the role of Na⁺-Ca²⁺ exchange in these processes.

POTASSIUM CHANNELS AND THE ACTION OF VASODILATORS

A number of substances dilate arteries by causing hyperpolarization. It has been suggested that the hyperpolarizing actions of the drugs cromakalim (BRL 34915), pinacidil, minoxidil, nicorandil, RP 49356 (Rhone-Poulenc), and diazoxide are caused by the opening of K channels (39, 40, 52, 85, 99, 132). An obvious mechanism by which hyperpolarization might cause vasorelaxation is by reducing Ca²⁺ entry through voltage-dependent Ca channels, but effects on intracellular Ca²⁺ uptake and release and via Na⁺-Ca²⁺ exchange have also been proposed (99). The nature of the K⁺ conductance pathway involved in hyperpolarization has been the subject of much work. Three channel types have been suggested: KATP (33, 99, 100, 119, 134), Ca²⁺-activated K (KCa) channels (55, 89), and delayed rectifier K channels (13). Much of the work on the type of K channel activated by hyperpolarizing vasodilators has been discussed recently (39, 40, 99, 132). Here, we will briefly discuss some of the work relating to these three channel types, giving particular attention to electrophysiological studies at the single-channel and whole cell level.

K channels can hyperpolarize at low levels of activation.

Very few K channels need open to produce a significant membrane hyperpolarization, since the resting input resistance of smooth muscle cells is high, in the order of 1–10 GΩ (1, 77). This is illustrated by a calculation based on a simple parallel conductance model. We assume that the Vm results from two parallel conductance pathways, a K⁺-selective conductance (Gk) representing the various K channels and a leakage pathway (Gl) approximating Cl⁻, Na⁺, and Ca²⁺ conductances, with a reversal potential of 0 mV. Vm will then be given by

\[ V_m = \frac{G_k}{G_k + G_l} E_K \]

where \( E_K \) is the K⁺ equilibrium potential, which we will take as -90 mV (67). Assuming an input resistance of 2 GΩ, corresponding to a conductance of 500 pS, and a resting potential of -55 mV, \( G_K \) will be 305 pS. Unitary conductances of K channels vary widely, but taking a typical value of 20 pS (91), the opening of one channel would hyperpolarize by 1.2 mV, while 20 or 50 channels would change Vm to -71 or -78 mV, respectively. This suggests that significant hyperpolarization may be achieved at low values of Popen. For example, if the membrane of a single smooth muscle cell contains 1,000 such channels, then increasing Popen from effectively 0 to 0.001 would hyperpolarize from -59.0 to -56.2 mV. This hyperpolarization could significantly reduce force (Fig. 3B and Ref. 92) and increase blood vessel diameter (26).

This vasodilation could lead to a rather substantial increase in blood flow, since flow is related to the fourth power of the radius. Therefore, low levels of K-channel activation could cause significant vasodilation.

KATP channels. Recently, KATP channels have been identified in arterial smooth muscle at the single-channel
level (78, 119). KATP channels were first described in cardiac muscle (95) and have since been reported in skeletal muscle (117), pancreatic β-cells (4, 38), and central neurones (5, 6). Many aspects of these channels have been reviewed recently (3, 107, 120). It is becoming clear that KATP channels form a family of channels differing to some extent between tissues, for example, in conductance, ATP sensitivity, and sensitivity to sulfonylurea blockers. Although these channels are defined by their sensitivity to intracellular ATP, ATP may not be the most important physiological modulator of their activity in many tissues. Channel Popen has been reported to be affected by a number of factors in addition to ATP, including ADP, GDP, GTP, and pH, and some of these also modulate channel sensitivity to ATP (3, 42, 44, 120). KATP channels can also be activated by neuropeptides in intact cells (44, 91, 119) with physiological levels of intracellular ATP and thus be made effectively insensitive to ATP. Phosphorylation appears to be required for the maintenance of channel activity in β-cells. The details of regulation may differ between tissues, especially since Popen is probably considerably higher at rest in β-cells than it is in muscle. The variation in channel activity between patches in smooth muscle and modulation by neuropeptides suggests that regulation of the channel is also complex in this tissue (91, 119).

A characteristic property of KATP channels is the reduction in their Popen that occurs as the concentration of ATP at the intracellular membrane face is increased. Figure 4A illustrates this property in a patch of membrane excised from an isolated smooth muscle cell from rabbit mesenteric artery, in which 1 mM ATP abolished channel openings. In smooth muscle, the stoichiometry of inhibition by ATP remains to be determined, but the channel is clearly highly sensitive to ATP, comparable to channels in excised patches from cardiac and skeletal muscle and β-cells, which show inhibitory constant (K_i) values in the range of 10–200 μM (3). In contrast, KATP channels reported in cortical and hypothalamic neurones are much less sensitive to ATP, with K_i in the 1–3 mM range (5, 6).

The unitary conductance of KATP channels of arterial smooth muscle was 135 pS in 60 mM [K+]_o/120 mM [K+]_i. It will certainly be much lower than this in physiological [K+]_o. Conductance is not such a useful measure under these conditions, in which the unitary current-voltage relation is strongly curved. Single K channels activated by calcitonin gene-related peptide (CGRP) (which causes a glibenclamide-sensitive hyperpolarization in cells from mesenteric artery) had unitary currents of ~3.0 pA at 0 mV in the physiological [K+]_o of 6 mM (Fig. 4B, Table 1).

The sulfonylurea antidiabetic compounds tolbutamide and glibenclamide have been shown to block KATP channels at the single-channel level in β-cells and cardiac muscle (110, 123, 127). These sulfonylureas appear to be specific blockers of KATP channels (3). Nevertheless, there seem to be considerable differences in potency between tissues, with β-cells being more sensitive than skeletal and cardiac muscle (31, 44, 49, 109). Substantial block of smooth muscle KATP channels in isolated patches occurs with 10–20 μM glibenclamide applied internally (119). Several other substances which are non-specific, in the sense that they block some other K channels as well, also block KATP channels with varying effectiveness. These include 4-aminopyridine, 9-aminoacridine, quinine, and Ba²⁺ (38, 50, 72, 104). The
channel is relatively insensitive to external tetraethylammonium (TEA+) which has a dissociation constant ($K_d$) $>7$ mM (31, 43).

Large-conductance $K_{Ca}$ channels. $K_{Ca}$ channels appear to be present in virtually every type of smooth muscle so far investigated. These channels are activated by [Ca$^{2+}$]$_i$ and blocked by external charybdotoxin (CTX) and TEA+. They are also voltage dependent, $P_{\text{open}}$ increasing quite steeply with depolarization. The properties of these channels in a variety of tissues have been reviewed recently (21, 80), and we have provided a brief summary for comparison with $K_{ATP}$ and delayed rectifier channels in Table 1. At present, the function of $K_{Ca}$ channels in arterial smooth muscle is not clear. They may be present at $V_m$ in arteries that exhibit tone, a condition in which [Ca$^{2+}$]$_i$ is elevated and $V_m$ is slightly depolarized. These channels may play an important role in the regulation of colonic smooth muscle $V_m$ during slow waves and acetylcholine activation (37).

Delayed rectifier channels. Delayed rectifier $K$ current has been studied at the whole cell level in smooth muscle cells from portal vein, and a few single-channel recordings have also been made (14, 82). The current is voltage dependent, activating with depolarization, and also shows time- and voltage-dependent inactivation. The current is blocked by 4-aminopyridine but is quite insensitive to TEA+. Single-channel recordings suggest that the channel is of low conductance in smooth muscle, $\approx 5$ pS (14; see also Table 1).

What kind of $K$ channel do hyperpolarizing vasodilators open? At the single-channel level, certain hyperpolarizing vasodilators have been shown to activate $K_{ATP}$ channels in insulin-secreting cells, cardiac myocytes, and arterial smooth muscle. Diazoxide activates $K_{ATP}$ channels in insulin-secreting cells (46, 124, 127), and cromakalim is also effective in some cell lines (47). Activation occurs against a background of channel inhibition by ATP. Cromakalim, pinacidil, nicorandil, and RP 49356 all activate $K_{ATP}$ channels in cardiac myocytes, both in on-cell and excised patches, and corresponding effects on whole cell currents are seen (2, 48, 49, 66, 109). Pinacidil, cromakalim, and RP 49356 did not affect inward or delayed rectifier $K$ channels in these cells (2, 48, 49). In patches excised from arterial smooth muscle, 1 $\mu$M cromakalim can activate $K_{ATP}$ channels in the presence of ATP (119; Fig. 4A). Cromakalim also activates $K_{ATP}$ channels from aortic smooth muscle incorporated into planar lipid bilayers (78).

Cromakalim and diazoxide have also been reported to activate large-conductance $K_{Ca}$ channels of vascular myocytes and aortic cells, either in native membrane or in planar lipid bilayers (55, 79, 126). However, other investigators have found no effect of cromakalim on $K_{Ca}$ channels (13).

The common theme in the functional pharmacology of hyperpolarizing vasodilators is the inhibition of their effects, whether measured as vasorelaxation in vitro or in vivo, as $^{86}$Rb efflux or as $K^+$ current, by a group of sulfonylurea compounds, notably glibenclamide which acts at $\approx 0.1$ mM (13, 33, 99, 100, 119, 134). The relative potency of different sulfonylureas in blocking cromakalim-induced $Rb^+$ efflux correlates well with their affinity for binding to $K_{ATP}$ channels of an insulin-secreting cell line (100, 101). Glibenclamide binding sites have also been identified in arterial smooth muscle (R. Kovaes, personal communication). The effects of various hyperpolarizing vasodilators have also been shown to be inhibited by phencyclidine, 4-aminopyridine, low (50-200 PM) concentrations of Ba$^{2+}$ (13, 119, 134), and millimolar concentrations of TEA$^+$ (13, 99, 134).

In contrast, the specific blockers, respectively, of small- and large-conductance $K_{Ca}$ channels, apamin and CTX, are without effect on relaxations produced by hyperpolarizing vasodilators (36, 99, 122, 131, 133, 134). External TEA$^+$ at 0.5-1 mM produces substantial block of large conductance $K_{Ca}$ channels (13, 70) but does not inhibit the effects of hyperpolarizing vasodilators (99, 119, 134), whereas 4-aminopyridine is a blocker of cromakalim-induced current but does not block $K_{Ca}$ channels (13).

Thus, although $K$-channel openers may activate $K_{Ca}$ channels under certain conditions (55, 79, 126), these channels do not appear to be involved in the functional effects of the compounds. The sensitivity of the actions of hyperpolarizing vasodilators to glibenclamide and other sulfonylureas, together with the pattern of action

<table>
<thead>
<tr>
<th>Agent</th>
<th>ATP-Sensitive $K$ Channels</th>
<th>$Ca^{2+}$ Activated $K$ Channels</th>
<th>Delayed Rectifier Channel (Vascular Smooth Muscle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>External TEA$^+$</td>
<td>Low-affinity block, $K_d$ $&gt;7$ mM (43)</td>
<td>Relatively high-affinity block, $K_d = 150-300$ nM (13, 70)</td>
<td>Very low-affinity block, $K_d &gt; 90$ mM (13)</td>
</tr>
<tr>
<td>Charybdotoxin</td>
<td>? No effect</td>
<td>High-affinity blocker, $K_d = 5-10$ nM (21, 80)</td>
<td>No effect (13)</td>
</tr>
<tr>
<td>Sulfonylureas, e.g., glibenclamide</td>
<td>Inhibition, $K_d &lt; 10$ nM (3, 119)</td>
<td>No effect (13)</td>
<td>No effect (13)</td>
</tr>
<tr>
<td>External Ba$^{2+}$</td>
<td>High-affinity block, $K_d &lt; 100$ nM (104)</td>
<td>Very low affinity, $K_d &gt; 10$ mM (21, 80)</td>
<td>No effect at 0.5 mM (67)</td>
</tr>
<tr>
<td>Voltage dependence</td>
<td>Weak (3, 117, 119, 120)</td>
<td>Strong (21, 80)</td>
<td>Strong (14)</td>
</tr>
<tr>
<td>Single-channel current at 0 mV in physiological $K^+$</td>
<td>3.0 pA (91)</td>
<td>5.0-5.4 pA (16, 70)</td>
<td>0.4-0.7 pA (14)</td>
</tr>
<tr>
<td>Intracellular ATP</td>
<td>Inhibits (3)</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Intracellular $Ca^{2+}$</td>
<td>No effect (118)</td>
<td>Activates (21, 80)</td>
<td>No effect</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to references. TEA$, tetraethylammonium; $K_d$, dissociation constant.
of other K-channel blockers, strongly suggests that a K\textsubscript{ATP} channel underlies the functional effects of these compounds (99). An alternative proposal is that cromakalim may activate delayed rectifier K channels, acting to remove their normal voltage dependence so that they open at the resting potential. Such channels are not blocked by glibenclamide, but it is proposed that this drug may interfere instead with the binding of cromakalim (13). However, because the actions of pinacidil, diazoxide, and RP 49356 are all glibenclamide sensitive, an action of the hyperpolarizing vasodilators on the delayed rectifier would imply that glibenclamide is interfering with the binding of a variety of chemically unrelated vasodilators, a possibility that seems unlikely. Furthermore, TEA\textsuperscript{+} blocks cromakalim-induced K\textsuperscript{+} currents (13) and dilations at concentrations (7 mM) below those needed to block delayed rectifier currents in portal vein. Minoxidil sulfate, which opens a glibenclamide-sensitive K channel, does not affect the delayed rectifier in portal vein (82).

In summary, we regard activation of K\textsubscript{ATP} channels as the most economical hypothesis for the mechanism of action of hyperpolarizing vasodilators at present. The demonstration that a particular channel can be activated by a drug does not show that it underlies the functional effects of these compounds. It is easier to exclude channels for which specific blockers are available. Thus the quantitative characterization of the actions of K-channel blockers, in particular glibenclamide as well as Ba\textsuperscript{2+}, TEA\textsuperscript{+}, and CTX on K\textsubscript{ATP}, K\textsubscript{Ca}, delayed rectifier, and inward rectifier channels in resistance arteries will be a necessary prerequisite to the final establishment of mechanism of actions of hyperpolarizing vasodilators. In addition, because activation of K\textsubscript{ATP} channels on cell would occur in the presence of high intracellular ATP, the ATP sensitivity of a channel might be reduced, as has been shown in insulin-secreting and cardiac cells so that they might not show high ATP sensitivity after activation. It is also not yet completely clear whether the mechanisms of action of hyperpolarizing vasodilators are the same in all tissues or for all compounds (99).

In addition to the drugs discussed above, a number of endogenous hyperpolarizing vasodilators may also act by opening the same K channel. Hyperpolarizations of arterial smooth muscle to vasoactive intestinal polypeptide (Fig. 5A), CGRP (91), and acetylcholine, which is thought to release a relaxing and a hyperpolarizing factor from the endothelium (125), are blocked by glibenclamide and Ba\textsuperscript{2+} (119). Glibenclamide completely reverses the hyperpolarization to these agents but gives a partial reversal of vasorelaxation (Fig. 5B), suggesting that vasodilators also act by an additional route, e.g., by increasing Ca\textsuperscript{2+} extrusion or sequestration, by directly closing Ca channels, or by shifting the free Ca\textsuperscript{2+}-steady-state force relationship. The suggestion that K\textsubscript{ATP} in arterial smooth muscle may be activated by peptide vasodilators has an interesting parallel in \beta-cell, in which gallatin and somatostatin have been shown to activate K\textsubscript{ATP} channels through a G protein (44). The metabolic sensitivity of K\textsubscript{ATP} channels might also provide a link between smooth muscle metabolism and blood flow. For example, it is conceivable that hypoxia-induced vasodilation may involve activation of K\textsubscript{ATP} channels.

CONCLUSIONS

We discuss recent evidence that vasoconstrictors can open voltage-dependent Ca channels. We suggest that vasoconstrictor activation of voltage-dependent Ca channels, along with other recent information on these channels, can account for many of the properties originally ascribed to ROCs. We propose that Ca\textsuperscript{2+} entry through voltage-dependent Ca channels is an important contributor to the regulation of tone in resistance vessels. Because Ca channels are strongly voltage dependent, the slightest change in \(V_m\) could have a substantial effect on Ca\textsuperscript{2+} entry. We propose that the voltage dependence of Ca channels underlies the steep membrane potential dependence of force (Fig. 3B and Ref. 92) and blood vessel diameter (26) and provides the link between membrane hyperpolarization and vasodilation. Furthermore,
we propose that \( K_{\text{ATP}} \) channel activation by drugs and neuropeptides may be a common mechanism of membrane hyperpolarization and vasodilation. The \( V_m \) of arterial smooth muscle cells in small arteries and arterioles must be tightly regulated in vivo or blood flow could fluctuate dramatically. In fact, it is conceivable that hypertension develops when this fine homeostatic regulation of \( V_m \) is disturbed.

**APPENDIX**

**Voltage Dependence of Steady-State \( P_{\text{open}} \)**

The basic description of channel kinetics stems from the following observations that have been confirmed with single-channel measurements. The channel can exist in one of two functional conformations, ion conducting (open) and nonconducting (closed). The transitions between these two conformations are rapid (\( \mu s \)) compared with the time spent in each (ms), and when the channel is open, ions permeate it at a nearly constant rate. Channels are also assumed to be independent of one another and essentially identical in their function. Finally, the kinetics of switching between these two conformations (gating) can be described both macroscopically (whole cells or tissues) and microscopically (single-channel currents) by the simple reactions used to describe linear chemical kinetics (see for example Ref. 65). Some of these principles and their implications for Ca-channel function will be outlined below.

At least three processes appear to determine whether or not a Ca channel is open. Ca channels can be regulated independent of \( V_m \) by cellular processes such as phosphorylation that determine whether or not they are able to open in response to \( V_m \) changes. We define the portion of Ca channels that can respond to \( V_m \) as “functional” and express the probability that this is so as \( P_{\text{funct}} \) (e.g., see Ref. 10). The dependence of Ca-channel opening on \( V_m \) is described by two processes, activation and inactivation. Membrane depolarization has two effects; it promotes a long-lived closed state (this process is called inactivation) and promotes the open state of the Ca channel (this process is called activation). Thus, for a channel to be open, it has to be functional, not inactivated, and activated. This can be expressed as the product of three probabilities so that

\[
P_{\text{open}} = (P_{\text{act}})(1 - P_{\text{inact}})(P_{\text{funct}}) \quad (A1)
\]

where \( P_{\text{open}} \) is the probability that the channel is open and \( P_{\text{act}} \) and \( P_{\text{inact}} \) are the probabilities that the channel is in the activated and inactivated states, respectively.

We will approximate activation and inactivation as voltage-dependent two-state processes, which can be represented functionally as resting \( \leftrightarrow \) activated and nonactivated \( \leftrightarrow \) inactivated. For each process, the probability at equilibrium will be given by a Boltzmann expression (see for example Hille, Ref. 65). This can be shown as follows. For the activation process, given by

\[
P_{\text{act}} = \frac{\alpha}{(\alpha + \beta)} \quad (A2)
\]

where \( \alpha \) and \( \beta \) are the transition rates between the two states, the equilibrium probability of being activated is

\[
P_{\text{act}} = \frac{\alpha}{(\alpha + \beta)} \quad (A2)
\]

These rate constants depend on the \( V_m \) gradient “felt” by the channel’s gates within the membrane’s interior. This voltage-dependent gating is thought to be due to charges on the gating structures that are energized by the membrane electrical field.\(^2\) If the reaction kinetics are given the standard form of linear kinetic reactions, the rate constants can be described quantitatively as reactions in which each functional state is an energy minimum, and an activation energy is required for transition to occur to another functional state. The application of \( V_m \) reduces or increases the energy difference between the minimum and the peak activation energy for the transition. For example, the rates of the reaction above can be written as

\[
\alpha = K \exp(-W_v + zdV_m/kT) \quad (A3)
\]

\[
\beta = K \exp(-W_v - z(1 - d)eV_m/kT) \quad (A4)
\]

where \( K \) is a constant, \( W_v \) and \( W_v^\prime \) are the activation energies for transition from the resting and activated states at 0 \( V_m \). \( z \) is the effective charge on the gate, \( d \) is the fraction of the membrane that is transversed for this charge to reach the activation energy maximum (usually 0.5), \( e \) is the elementary charge unit, \( k \) is the Boltzmann constant, and \( T \) is the temperature. Functionally, these equations indicate that \( \alpha \) increases exponentially as \( V_m \) becomes less negative, while \( \beta \) decreases exponentially. Lumping the constant factors, the quantitative description of these two rates becomes

\[
\alpha = K_1 \exp(\frac{V_m}{kT}) \quad (A5)
\]

\[
\beta = K_1 \exp(-\frac{V_m}{kT}) \quad (A6)
\]

Substituting into Eq. A2 above, \( P_{\text{act}} \) is thus given by

\[
P_{\text{act}} = \frac{1}{1 + \exp(-\frac{V_m}{kT})} \quad (A7)
\]

where

\[
k_1 = kT/ze \quad (A8)
\]

\[
V_0.5 = k_1(W_v - W_v^\prime) \quad (A9)
\]

Steady-state activation gating can thus be specified by a single Boltzmann relationship in which the two free parameters \( V_0.5 \) and \( k_1 \) can be determined experimentally. The degree of inactivation can be specified similarly.

In the range of potentials in which we are interested here, however, the \( V_m \) is likely to be substantially more negative than the half-maximal voltage for either steady-state activation or inactivation. In this case, \( P_{\text{inact}} \) approaches 1, and the relationship given by \( P_{\text{open}} \) above can be approximated by a single exponential. Therefore, the final probability that a Ca channel is functioning in this range of \( V_m \) values can be approximated as

\[
P_{\text{open}} \propto A \exp(\frac{V_m}{kT}) \quad (A10)
\]

where

\[
A = P_{\text{funct}} \exp(\frac{V_0.5}{kT}) \quad (A11)
\]

**NOTE ADDED IN PROOF**

J. Daut et al. (Hypoxia dilation of coronary arteries is mediated by ATP-sensitive potassium channels. Science Wash. DC 247: 1341–1344, 1990) have provided evidence that activation of another subunit of skeletal and cardiac muscle Ca channel as well as voltage-dependent Na and K channels senses the \( V_m \) (called the voltage sensor, Ref. 30). Although the arterial smooth muscle Ca channel has not yet been purified or cloned, presumably it is structurally related to the cardiac muscle Ca channel. In any case, the voltage sensor appears to be a conserved part of voltage-dependent Ca, Na, and K channels (30) and thus unlikely to be substantially different in this tissue.
tion of \( K_{\text{ATP}} \) channels mediates hypoxia-induced coronary artery dilation.

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REFERENCES

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