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Mechanisms of Drug-induced Vasodilation*

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Several cardiovascular disorders, most notably hypertension, angina and heart failure, are commonly treated with vasodilators. These drugs improve the functioning of the cardiovascular system without treating the cause of the disease, which in most cases is unknown. The clinically used drugs belong to a large, heterogeneous group of agents that share the ability to dilate blood vessels in-vivo. Their beneficial effects result principally from the dilation of arterial resistance vessels, leading to reduced peripheral resistance, or dilation of the venous capacitance vessels, reducing venous return to the heart. There are many different types of vasodilator, which display varying degrees of selectivity for different vascular beds and have distinct clinical profiles. This is reflected in the varied therapeutic applications of the different vasodilator types. Thus, hypertension may be treated with an arteriole dilator, such as the calcium antagonist nifedipine, whereas angina pectoris responds to glyceryl trinitrate, which acts predominantly on the veins. The effectiveness of a vasodilator in treating a particular condition depends on many factors in addition to the clinical profile of the disease and drug. Variability in response among individuals is a well-known phenomenon that has particular consequences in the treatment of essential hypertension. Hypertensives are usually free of any noticeable symptoms and feel well. Thus, despite the importance of lowering their blood pressure, hypertensives are often unwilling to keep taking drugs that produce unpleasant side-effects. Although a large number of different vasodilators are available, they all produce side-effects that may or may not be well tolerated, depending on the individual.

A multitude of factors normally regulate vessel diameter and tone, which depends ultimately on the contractile state of the smooth muscle in the medial layer of the blood vessel wall. Vessels constrict when the smooth muscle contracts, either in response to nerve stimulation or the action of local or blood-borne hormones. Factors that oppose contraction also play a vital role. In particular, it became clear recently that the endothelial cells lining the inside of blood vessel walls release relaxing factors that are important for the local control of vascular muscle tone and vessel diameter. Endothelial cells secrete a number of vasoactive substances, the best studied of which is the so called endothelium-derived relaxing factor, or EDRF, recently identified as nitric oxide (NO). Studies in man have concluded that, in some vascular beds, there is a basal dilator tone maintained by the continuous release of NO from the endothelium. Thus, loss of endothelial function, with consequent loss of NO-mediated vasodilation, has been suggested as a possible cause of essential hypertension.

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Vasodilating drugs can either act directly on the vascular smooth muscle to cause its relaxation, or they can act indirectly either by stimulating the release of an endogenous dilator, such as EDRF, or by inhibiting the action of an endogenous vasoconstrictor. This overview concentrates on the mechanisms of action of the directly-acting vasodilators, which include several different types of drug with quite disparate effects. The purpose of this presentation is to outline our present understanding of the mechanisms of action of these drugs at the cellular level. The variety of mechanisms underlying the effects of directly-acting vasodilators reflects the multiple pathways that normally participate in the smooth muscle cell to regulate and maintain tension.

The Normal Regulation of Vascular Tone

Calcium is the primary regulator of tension in vascular smooth muscle. When the concentration of free Ca^{2+} rises in the cell, it triggers the activation of the Ca^{2+}-calmodulin-dependent protein kinase, which in turn activates myosin light-chain kinase to phosphorylate myosin light chains. This enables actin and myosin to interact and generate active tension. Additional processes contribute to the maintenance of vascular tone. Tension is well maintained in the continued presence of an agonist, even though the cytoplasmic Ca^{2+} concentration falls to a lower sustained level following the initial Ca^{2+} peak (Karaki 1989; Nishimura & van Breemen 1989). Thus, the force: Ca^{2+} ratio increases during sustained tension, implying that Ca^{2+}-sensitizing mechanisms contribute to agonist-induced tone. Neither the physiological importance nor the underlying mechanisms of Ca^{2+} sensitization are clear. Protein kinase C, which is activated physiologically by diacylglycerol, a product of agonist-activated phospholipase C activity, has been implicated as a possible intracellular mediator (Nishimura & van Breemen 1989).

Vasoconstrictors raise the concentration of free Ca^{2+} in the cytosol by activating or stimulating several pathways, as outlined in Fig. 1. Some of the Ca^{2+} originates from the extracellular space, and is transported into the cell through Ca^{2+}-permeable channels in the cell membrane. There are two distinct types of channel for Ca^{2+} entry, receptor-operated and voltage-operated channels (Bolton 1979). The receptor-operated channels are opened by a vasoconstrictor agent, such as noradrenaline or ATP, binding to specific membrane receptors (Benham & Tsien 1987; Byrne & Large 1988; Xiong et al 1991). These channels do not show a high selectivity for Ca^{2+}, but are permeable to several cations. The ATP-gated channel displays a permeability ratio for Ca^{2+} to Na^{+} of about 3:1, so that Ca^{2+} carries only about 10% of the

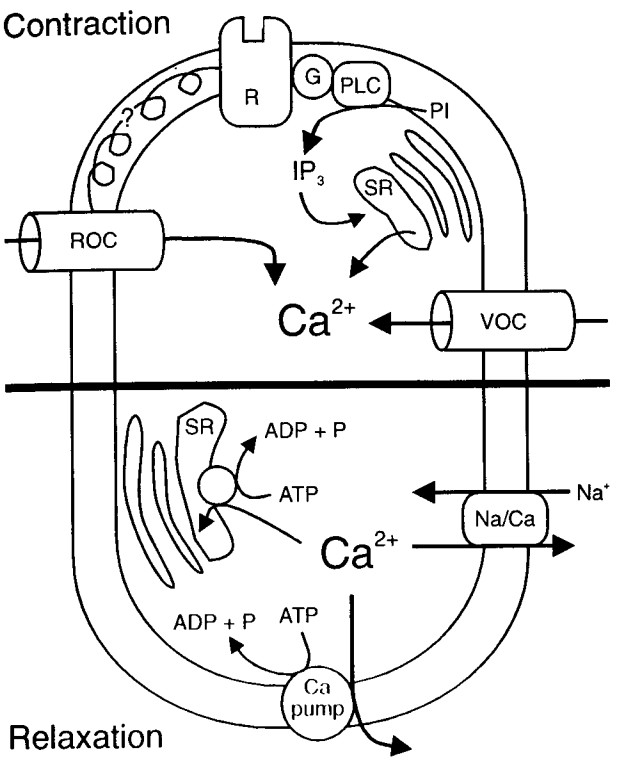

FIG. 1. Normal regulation of intracellular Ca^{2+} concentration and tension in vascular smooth muscle cells. R: receptor, G: GTP-binding protein, PLC: phospholipase C, ROC: receptor-operated channel, VOC: voltage-operated channel, Na/Ca: Na/Ca exchanger.

total current through the channel (Benham & Tsien 1987). These channels do, nevertheless, admit sufficient Ca^{2+} to elevate significantly the intracellular Ca^{2+} concentration and contribute to contraction (Benham 1989). The mechanism of coupling between the receptor and channel has not been clearly elucidated. Electrophysiological studies of ATP-activated channels in membrane patches from arterial muscle, point to a direct gating of the channel by the agonist (Benham & Tsien 1987). It is also possible that the channel and receptor couple through a GTP-binding protein (Xiong et al 1991), a mechanism that is employed by a number of other, apparently direct, receptor-channel interactions. The channels activated by ATP are not necessarily the same as those that couple to the receptors employed by other vasoconstrictors (Byrne & Large 1988). Some differences are apparent, for example, the responses of arterial smooth muscle cells to noradrenaline appear to depend on the presence of an intracellular mediator (Amèdèe et al 1990).

Voltage-operated Ca^{2+} channels are selectively permeable to Ca^{2+}. The primary trigger that opens them is membrane depolarization, although their probability of opening in response to depolarization may be modulated by vasoactive agents. In the electrically quiescent, large, elastic arteries such as aorta, the membrane depolarization that leads to opening of the voltage-dependent Ca^{2+} channels is brought about by agonist-induced activation of receptor-operated channels. The voltage-operated channels are particularly important in spontaneously active vascular muscles, such as portal vein, where they contribute to basal tension in the absence of agonist (Bolton 1979). In these vessels, spontaneous action potentials, which generate spontaneous tension transients, depend upon the opening of voltage-operated Ca^{2+} channels.

Some of the Ca^{2+} for contraction derives from the sarcoplasmic reticulum, which behaves as an intracellular storage site for Ca^{2+} (Somlyo 1985). Release of Ca^{2+} from these stores can be triggered by inositol 1,4,5 trisphosphate (IP_3), which is formed in the cytosol from the breakdown of phosphatidylinositol (PI), following the binding of an agonist to its receptor on the cell surface. The involvement of IP_3-induced Ca^{2+} release in agonist-induced tension was confirmed by studies on permeabilized vascular muscle, where the intracellular environment could be controlled (Somlyo et al 1988). It was found that following rapid intracellular application of IP_3, tension developed with a much shorter latency than that following rapid extracellular application of the α-adrenergic agonist, phenylephrine. Thus, agonist-binding to α-adrenoceptors is followed, after a delay, by IP_3 production, Ca^{2+} release and then contraction. Ca^{2+} release from the sarcoplasmic reticulum may also be triggered by Ca^{2+} entry (Clapp & Gurney 1991a), resulting in a positive feedback loop that might amplify the rise in cytoplasmic Ca^{2+}.

Vascular muscle relaxes when intracellular Ca^{2+} falls below a threshold level. The removal of Ca^{2+} from the cytoplasm is brought about by a combination of Ca^{2+} accumulation into the sarcoplasmic reticulum and extrusion from the cell across the plasma membrane, as illustrated in Fig. 1. The major route of Ca^{2+} removal is probably the uptake of Ca^{2+} into the sacroplasmic reticulum (Kargacin & Fay 1991), which is accomplished by a Ca^{2+}, Mg^{2+}-ATPase (Ca^{2+} pump) that undergoes Ca^{2+}-dependent phosphorylation (Sumida et al 1984). The activity of the pump is regulated by phospholamban, a protein that is localized to the sarcoplasmic reticulum (Ferguson et al 1988) and is modulated by cyclic nucleotide-dependent phosphorylation (Eggermont et al 1988a). The plasma membrane contains a distinct Ca^{2+}, Mg^{2+}-ATPase, which extrudes Ca^{2+} from the cell (Eggermont et al 1988b). This ATPase binds calmodulin so that its activity is stimulated when cytoplasmic levels of Ca^{2+} rise. Smooth muscle membrane preparations have also been shown to contain a Na^{+}-Ca^{2+} exchanger (Grover et al 1983). This may contribute significantly to Ca^{2+} extrusion in some blood vessels (Aaronson et al 1991), but it is still debated how much it contributes in others. The relative contribution of each of these Ca^{2+}-removal processes may well vary among different vascular beds.

The variety of pathways that contribute to Ca^{2+} homeostasis and the maintenance of tone in vascular smooth muscle, means that there are many potential targets for drug interactions that can produce vasodilation. It is therefore not surprising that a large number of vasodilators with disparate actions exist. Some drugs, e.g. sodium nitroprusside, cause a general vasodilation, being equally effective on the arterial and venous circulations. However, others show a selective action on specific vascular beds. This is possible, in part, because the relative contribution that each of the Ca^{2+}-regulating pathways makes to muscle tension varies among different vascular beds, and with the contractile stimulus.

Calcium Antagonists

The main groups of calcium antagonist used clinically comprises the 1,4-dihydropyridines, the phenylalkylamines and the benzothiazepines, of which nifedipine, verapamil and diltiazem are representative examples. These drugs all act primarily by blocking Ca^{2+} influx through voltage-gated Ca^{2+} channels. Most cells contain voltage-operated Ca^{2+} channels, through which Ca^{2+} enters to trigger intracellular events. However, several channel subtypes, representing distinct molecular forms exist and they are differentially distributed to different tissues. The therapeutic Ca^{2+} antagonists block the subtype that predominates in cardiac and smooth muscle. Consequently, the effects of these drugs in-vivo are mainly on the cardiovascular system. All three groups of Ca^{2+} antagonist will block the Ca^{2+} channels found in both the heart and blood vessels. They do, however, differ in their selectivity of action towards the two tissues. Verapamil is cardioselective, its main effect being to inhibit atrioventricular conduction; it thereby suppresses supraventricular tachycardias. By contrast, the dihydropyridine Ca^{2+} antagonists are more powerful vasodilators, and relax vascular muscle at concentrations having little effect on the heart. Diltiazem is intermediate in its action, affecting the heart at doses required to produce vasodilation. Tissue selectivity is apparent, even within a group of drugs. Different dihydropyridine molecules show selectivity for different vascular beds. For example, nimodipine acts more potently on the cerebral vasculature than on peripheral vessels (Towart 1981) and nisoldipine is relatively selective for coronary vessels (Godfraind et al 1992). Recent studies of Ca^{2+} antagonists have attempted to explain the mechanistic basis underlying these tissue selectivities.

Cloning studies have shown the Ca^{2+} channel to be comprised of 5 subunits, α_1, α_2, β, γ and δ. The α_1 subunit is the main channel-forming subunit and it contains the binding sites for all of the Ca^{2+} antagonist drugs (Koch et al 1990). This subunit is very similar in the Ca^{2+} channels from heart and aorta (Koch et al 1990). The roles of the other subunits are not yet clear, but they are not needed for channel formation or drug binding. Nevertheless, they may influence the properties of the channel or its modulation by drugs (Hullin et al 1992; Itagaki et al 1992). Diltiazem, verapamil and nifedipine are known to bind at three distinct sites on the α_1 subunit, and these sites interact allosterically (Catterall et al 1988). Thus, the binding of verapamil to the channel reduces its affinity for dihydropyridines, while diltiazem binding has the opposite effect. Several studies have located the phenylalkylamine receptor at the intracellular surface of the channel (Hescheler et al 1982; Striessnig et al 1990), whereas the dihydropyridine (Kass et al 1991; Striessnig et al 1991) and benzothiazepine (Hering et al 1993) receptors appear to be present close to the extracellular surface. The location of the Ca^{2+}-antagonist binding site, in relation to the channel-forming domains of the protein, probably determines the ability of a drug to slow atrioventricular conduction. Phenylalkylamines are particularly effective at this, because their interaction with Ca^{2+} channels is use-dependent. This property was clearly demonstrated in a study of the current flowing through Ca^{2+} channels in isolated heart cells, where the actions of verapamil and D600 (methoxy verapamil) were compared directly with diltiazem and nitrendipine (Lee & Tsien 1983). Whereas block by D600 was entirely use-dependent, nitrendipine showed essentially no use-dependence and diltiazem was intermediate in its action. The property of use-dependence implies that a drug can gain access to its blocking site only when the channels have been opened. Thus, the phenylalkylamines may only be able to reach the binding site on the cytoplasmic side of the channel once the channel has been opened. The significance of this property is that in rapidly-firing tissue, as in the case of a supraventricular tachycardia, block of the channels by verapamil would be enhanced relative to the other Ca^{2+} antagonists, because Ca^{2+} channels would be opening more frequently, allowing greater interaction between verapamil and the channel.

Membrane potential plays an important role in modulating the binding of dihydropyridine Ca^{2+} antagonists to Ca^{2+} channels. The inhibition of Ca^{2+}-channel activity increases with depolarization in arterial (Bean et al 1986) and venous (Yatani et al 1987) smooth muscle. As first elaborated in heart (Bean 1984; Gurney et al 1985), the voltage dependence can be explained by the modulated-receptor hypothesis (Hille 1977), in which drugs bind with higher affinity to inactivated channels than to closed (resting) or open channels. Thus, at negative potentials (below around -45 mV), where few channels are normally inactivated, block by dihydropyridines is relatively small. At more depolarized potentials, the proportion of Ca^{2+} channels in the inactivated state increases and block is enhanced. It is likely that depolarization induces a conformational change in the channel that favours dihydropyridine binding. This may help to explain some of the tissue selectivities of dihydropyridine Ca^{2+} antagonists. As in cardiac muscle, the Ca^{2+} channel in smooth muscle characteristically opens at membrane potentials positive to -40 mV and then gradually inactivates during maintained depolarization (Clapp & Gurney 1991b). Since most vascular muscles have a resting potential more negative than -40 mV, in resting, fully relaxed blood vessels the Ca^{2+} channels would be mainly closed. However, with increasing depolarization, brought about in blood vessels by, for example, increased transmural pressure (Harder 1984), vasoconstrictors such as noradrenaline (Büllbring & Tomita 1987) or elevated extracellular K^+ concentrations, there would be an increased probability of Ca^{2+} channels opening and subsequently inactivating. In this situation, dihydropyridines become more effective (Fig. 2). Similarly, vascular muscles that display spontaneous electrical activity characterized by Ca^{2+}-influx-dependent, rhythmic depolarizations would be more strongly blocked by drugs that interact with inactivated Ca^{2+} channels.

The highest affinity for the 1,4-dihydropyridines is found in the Ca^{2+} channels of vascular muscle. It is likely that the affinity for these agents depends not just on the α_1-subunit, in which two specific regions differ between heart and vascular muscle, but also on the additional subunits that make up the complete channel protein. The influence of β-subunits derived from heart (Hullin et al 1992) and skeletal muscle (Itagaki et al 1992) has been studied by co-expressing them in *Xenopus* oocytes with the α_1-transcript from aorta (Itagaki et al 1992) or heart (Hullin et al 1992; Itagaki et al 1992). When a β-subunit was present, the amplitude of the expressed

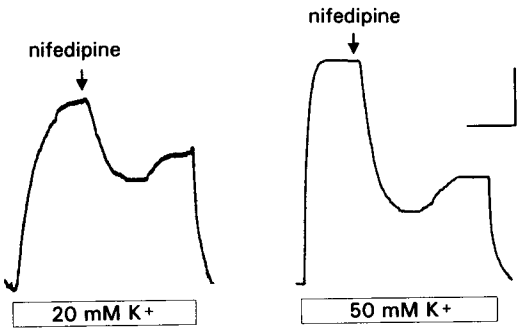

FIG. 2. The relaxant effect of nifedipine becomes more pronounced when smooth muscle is depolarized. The tension developed by an isolated strip of rabbit main pulmonary artery was measured and nifedipine (0·5 μM) perfused across the tissue for 30 s. Nifedipine relaxed the tissue when it was applied in the presence of 20 mM K^+, which causes a moderate depolarization and muscle contraction. Its effect was enhanced when it was applied in the presence of 50 mM K^+, which produces a larger depolarization and contraction. Calibration bars correspond to 10 min and 125 mg (20 mM K^+) or 10 min and 250 mg (50 mM K^+).

current was enhanced, and the percentage increase in the current produced by the dihydropyridine agonist Bay K 8644 was reduced. A possible modulatory role for the β-subunit is also suggested by the finding that the expression of its various isoforms is tissue specific (Hullin et al 1992). There is also evidence of a modulatory role for the α_2-subunit (Hullin et al 1992; Itagaki et al 1992).

Although progress has been made towards understanding the mechanistic basis for the tissue selectivities shown by different Ca^{2+} antagonists, we still do not know why some dihydropyridines show selectivity for specific vascular beds. Part of the explanation may lie in the variation in resting potentials seen in different blood vessels, or in the sources of Ca^{2+} utilized by a particular vascular muscle for contraction. In some blood vessels, receptor-operated channels or intracellular Ca^{2+} stores may provide a more important source of Ca^{2+} than the voltage-operated channels. However, these explanations cannot account for the selective effect shown by nimodipine towards cerebral vessels, or the coronary selectivity of nisoldipine, since these selectivities are not shared by all dihydropyridines. Perhaps the answer lies in varying subunit compositions of the Ca^{2+} channel in different vascular beds. So far, the only vascular tissue to have been used as a source for cloning Ca^{2+}-channel proteins is rat aorta. Our full understanding of selectivity may have to wait until more subunits have been cloned and sequenced from different blood vessels, and their functional properties established.

Potassium-channel Openers

Since the opening of Ca^{2+}-channels requires membrane depolarization, then agents that produce hyperpolarization will cause Ca^{2+} channels to close, which in turn will reduce Ca^{2+} influx and promote muscle relaxation. Hyperpolarization of the smooth muscle cell membrane can be produced by agents that open K^+ channels, thereby enhancing K^+ efflux from the cell. Drugs thought to produce vasodilation in this way include diazoxide and minoxidil sulphate, which have been in clinical use for some time although their mechanisms of action are only now being appreciated. Newer drugs that work through this mechanism include cromakalim (or its active enantiomer levcromakalim), pinacidil and nicorandil, although the latter drug also has properties in common with the organic nitrates. Substantial evidence that these drugs open K^+ channels to produce hyperpolarization has been provided by a number of different experimental approaches, including electrophysiological and ion-flux studies (reviewed in Cook & Quast 1990). A characteristic property of these drugs is that they effectively inhibit vascular muscle contraction brought about by a moderate increase in the extracellular K^+ concentration (to <40 mM), but they are ineffective when the K^+ concentration is raised to higher levels (Fig. 3). This is because at high K^+ concentrations, the membrane potential of the smooth muscle cell is close to the K^+ equilibrium potential, where net flux of K^+ and therefore hyperpolarization is prevented. This property distinguishes K^+-channel openers from Ca^{2+} antagonists, which show the opposite behaviour (Fig. 2).

Recent work has focussed on establishing the nature of the K^+ channel targeted by the K^+-channel openers. Patch-clamp experiments have identified a number of K^+-channel types in arterial and venous smooth muscle that are influenced by drugs of this kind. These channels include a delayed rectifier (Beech & Bolton 1989), large (Gelband et al 1989; Klöckner et al 1989) and small (Kajioka et al 1990) conductance channels that normally depend on intracellular Ca^{2+} for their activation, and a variety of K^+ channels inhibited by intracellular ATP (Standen et al 1989; Kajioka et al 1990, 1991; Miyoshi et al 1992). It has proved to be surprisingly difficult to determine precisely which channel is responsible for the hyperpolarizing action of the drugs. Some insight into this was provided recently by experiments showing that the magnitude of the hyperpolarization produced in rabbit pulmonary arterial smooth muscle cells by levcromakalim was dependent on the intracellular concentration of ATP (Clapp & Gurney 1993). The hyperpolarization was accompanied by an increased K^+ conductance, the magnitude of which showed a similar dependence on ATP.

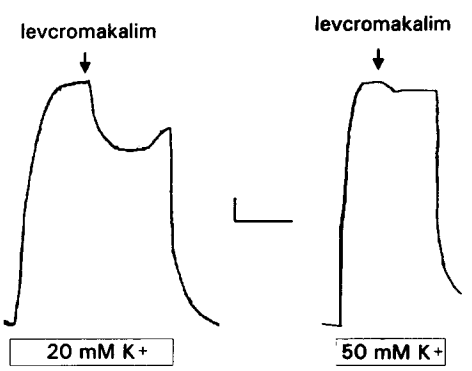

FIG. 3. The relaxant effect of levcromakalim becomes less pronounced as the extracellular K^+ concentration is raised. The tension developed by an isolated strip of rabbit main pulmonary artery was measured and levcromakalim (10 μM) perfused across the tissue for 1 min. The drug caused a pronounced relaxation when it was applied in the presence of 20 mM K^+, which produces moderate depolarization of the smooth muscle. It had little effect when applied in the presence of 50 mM K^+, when the membrane potential of the smooth muscle cells would be close to the K^+-equilibrium potential. Calibration bars correspond to 10 min and 90 mg (20 mM K^+) or 10 min and 250 mg (50 mM K^+).

The channels underlying this conductance increase may well be involved in the normal regulation of membrane potential in vascular smooth muscle, since simply varying the intracellular ATP concentration, in the absence of K^+-channel openers, modulates the membrane potential and conductance (Clapp & Gurney 1992). Furthermore, in cells depleted of ATP, there is a background K^+ current that can be blocked by rapidly reintroducing ATP into the cell by photolysis of caged ATP. Membrane depolarization accompanies the block of this current.

The accumulating evidence points to an ATP-sensitive K^+ (K_{ATP}) channel as the one responsible for the pharmacological effects of K^+-channel openers. The precise properties of the channel may vary in different vascular beds, since there are reports of K_{ATP} channels with a small conductance in some blood vessels (Kajioka et al 1990, 1991; Miyoshi et al 1992) but with a larger conductance in others (Standen et al 1989). Support for the involvememt of K_{ATP} channels also comes from work with the sulphonylurea drug, glibenclamide, a selective inhibitor of K_{ATP} channels in the pancreas and heart. Glibenclamide reverses the vasodilation and hyperpolarization produced by K^+-channel openers (Standen et al 1989; Cook & Quast 1990; McPherson & Angus 1991; Clapp & Gurney 1993) and blocks the ATP-sensitive K^+ channels activated by K^+-channel openers in membrane patches from vascular muscle (Standen et al 1989; Kajioka et al 1991; Miyoshi et al 1992). In contrast, the vasodilation and hyperpolarization produced by K^+-channel openers is relatively insensitive to inhibitors of Ca^{2+}-activated K^+ channels (Cook & Quast 1990). Furthermore, ATP depletion (Clapp & Gurney 1992, 1993) or metabolic inhibition (Silberberg & Van Breemen 1992; Noack et al 1992) of vascular smooth muscle cells activates a K^+ current, which has similar properties to the current activated by levcromakalim and is blocked by glibenclamide, but not by inhibitors of Ca^{2+}-activated K^+ channels. Thus, the vasodilating effects of K^+-channel openers can largely be explained by an action on K_{ATP} channels, although at higher concentrations they may have additional effects (Cook & Quast 1990; Noack et al 1992; Clapp & Gurney 1993).

Hyperpolarization may be employed by some endogenous agents to bring about vasodilation. The endothelium of a number of arteries releases a substance that hyperpolarizes vascular smooth muscle cells (Tare et al 1990; Brayden 1990). The agent responsible for hyperpolarization may be NO in some arteries (Tare et al 1990) but not in others (Feletou & Vanhoutte 1988; Chen et al 1988; Brayden 1990). Thus the existence of a separate endothelium-dependent hyperpolarizing factor (EDHF) has been postulated. Although it may not be important for endothelium-dependent relaxation in some blood vessels (Feletou & Vanhoutte 1988; Chen et al 1988), EDHF clearly plays a role in cerebral blood vessels (Brayden 1990; Brayden et al 1991), uterine artery (Tare et al 1990) and femoral veins (Nagao & Vanhoutte 1991). Endothelium-dependent hyperpolarization probably involves an increase in smooth muscle K^+ conductance, because it is accompanied by an increase in ^{86}Rb efflux (Chen et al 1988). An effect of EDHF on the membrane Na^+, K^+-ATPase has been suggested (Feletou & Vanhoutte 1988), but the majority of electrophysiological evidence supports the involvement of K^+ channels (Tare et al 1990; Brayden et al 1991). Since glibenclamide can reverse the acetylcholine-induced hyperpolarization in cerebral arteries, EDHF has been proposed to work by activating K_{ATP} channels (Standen et al 1989; Brayden et al 1991), although other studies argue against this (McPherson & Angus 1991). The effects of other vasodilators, such as vasoactive intestinal peptide (Standen et al 1989), calcitonin gene-related peptide (Nelson et al 1990) and prostacyclin (Siegal et al 1990), which act directly on smooth muscle, are thought to be mediated in part through membrane hyperpolarization. These agents may activate K_{ATP} channels (Nelson et al 1990; Brayden et al 1991), possibly via a G-protein or generation of intracellular messengers. Thus, it seems possible that the channels underlying the hyperpolarizing action of K^+-channel openers may be employed normally by endogenous vasodilators to regulate blood vessel tone.

Nitroprusside and Organic Nitrates

Sodium nitroprusside is a powerful vasodilator that is used in treating hypertensive emergencies and severe heart failure. It dilates arteries and veins, thereby reducing both peripheral resistance and venous return. The organic nitrates, including glyceryl trinitrate and isosorbide mono and dinitrates, are used primarily to treat angina pectoris, but also to treat congestive heart failure. These drugs can influence all parts of the vascular system, but they are more potent dilators of veins than of arteries (reviewed in Ahlner et al 1991). The main beneficial effect of the nitrates in angina pectoris is dilation of the large veins, leading to increased venous capacitance and reduced preload, but reduced peripheral resistance also contributes. Although sodium nitroprusside and the organic nitrates display a different spectrum of effects in-vivo and they have different clinical uses, the mechanisms underlying their actions are basically the same. Both types of drug can release free NO, which then acts to relax vascular smooth muscle in the same way as NO released endogenously from the endothelium. The different effects of the drugs probably reflect differences in the way that NO is produced from the parent compound. Whereas NO is liberated spontaneously from sodium nitroprusside in solution (Feelisch & Noack 1987), its release from the organic nitrates depends on an interaction between the drug and an SH-containing molecule (Ahlner et al 1991). The exact mechanism of nitrate degradation is not yet clear, but there is evidence for both enzymic (Ahlner et al 1991) and non-enzymic pathways (Feelisch et al 1988). This interaction appears to take place in or near the target smooth-muscle cell (Ahlner et al 1991), and may involve SH-groups on cell proteins.

Vasodilation induced by sodium nitroprusside or the organic nitrates is correlated with an elevation of the cGMP levels in vascular muscle (Katsuki et al 1977; Rapoport et al 1985). There is substantial evidence that intracellular cGMP mediates the effects of these drugs, as well as the effects of the endogenous vasodilators NO (Ignarro et al 1986) and atrial natriuretic peptide (ANP) (Rapoport et al 1985). In each case, the elevation of cGMP precedes relaxation, and inhibitors of guanylyl cyclase prevent the drug-induced relaxation while inhibitors of cGMP metabolism potentiate it. Furthermore, relaxation can be produced by membrane-

permeant analogues of cGMP (Francis et al 1988), and by direct intracellular application of cGMP in intact arterial muscle (Gurney 1993). Inhibitors of the cGMP-metabolizing enzyme, phosphodiesterase, also cause vasodilation (Wood & Owen 1989). Examples of phosphodiesterase inhibitors include the non-selective methyl xanthines, such as caffeine and theophylline. Amrinone and milrinone, which were introduced for the treatment of heart failure, are selective inhibitors of the phosphodiesterase III isoenzyme (Wetzel & Hauel 1988). The main effect of these drugs is to elevate intracellular levels of cAMP, which is also a mediator of smooth muscle relaxation. cAMP is thought to mediate their therapeutic actions, which include enhanced cardiac contractility and vasodilation, but elevation of cGMP levels could contribute.

There are two distinct guanylyl cyclase enzymes, found in the particulate and soluble fractions of cell homogenates, which catalyse the formation of cGMP in vascular muscle (Yuen & Garbers 1992). The particulate enzyme is the receptor for ANP. It is a membrane-spanning protein, which contains the receptor at the extracellular side and guanylyl cyclase activity on the cytoplasmic side (Yuen & Garbers 1992). The soluble enzyme is restricted to the cytosol. It is stimulated by NO and responds to NO-containing compounds such as sodium nitroprusside and the organic nitrates (Böhme et al 1984). The mechanisms linking the rise in cGMP levels to vasodilation have not yet been resolved, although many different actions of cGMP have been reported. It is generally believed that the effects of cGMP are mediated through cGMP-dependent protein (G) kinase, because a number of smooth-muscle cell proteins are phosphorylated by the kinase (Baltensperger et al 1990). In support of this, agents known to stimulate G kinase, such as 8-bromo-cGMP and 8-(4-chlorophenylthio)-cGMP relax vascular muscle (Francis et al 1988). Furthermore, the ability of 8-bromo-cGMP and ANP to reduce cytoplasmic Ca^{2+} levels in rat cultured aortic cells was correlated with levels of G-kinase in the cultures (Cornwell & Lincoln 1989). Kinase-independent effects, which are known to occur in other cell types, cannot, however, be ruled out.

The clearest effect of cGMP-dependent vasodilators in vascular smooth muscle is to lower the cytoplasmic free Ca^{2+} concentration (Morgan & Morgan 1984; Kai et al 1987; Rashatwar et al 1987; Karaki et al 1988). Several lines of evidence suggest that a reduction of Ca^{2+} transport into the smooth muscle cell contributes to this effect. For example, nitrovasodilators and cGMP analogues attenuate the Ca^{2+} influx and rise in intracellular Ca^{2+} concentration (Kai et al 1987; Cornwell & Lincoln 1989) induced by membrane depolarization and agonist stimulation. An action similar to that of the Ca^{2+} antagonist drugs was confirmed recently by the finding that sodium nitroprusside suppressed the Ca^{2+}-channel current recorded from rabbit isolated pulmonary arterial cells (Clapp & Gurney 1991b). The involvement of an intracellular messenger in the response was suggested by its relatively slow time-course, the maximum effect being achieved only after many seconds, and by its apparent requirement for intracellular ATP. Thus, the Ca^{2+} antagonist-like action may be mediated through G-kinase, which is known to phosphorylate membrane proteins in vascular muscle (Baltensperger et al 1990). One of these proteins may be involved in interactions between the plasma membrane and cytoskeleton (Baltensperger et al 1990), but the others have yet to be identified. Perhaps one is in some way associated with the Ca^{2+} channel. The purified Ca^{2+} channel from skeletal muscle is phosphorylated in-vitro by cGMP-dependent protein kinase (Jahn et al 1988), although comparable information is not available for the vascular channel. Whatever the mechanism, inhibition of voltage-gated Ca^{2+} channels cannot fully account for vasodilation induced by NO-containing drugs. This is because, as illustrated in Fig. 4a, they are much less effective at inhibiting contractions induced by membrane depolarization than by agonist stimulation. The opposite is true of the classical Ca^{2+} antagonists (Fig. 4b).

In some blood vessels, NO and NO-containing vasodilators have been found to cause hyperpolarization of the smooth-muscle cell membrane (Ito et al 1978; Tare et al 1990). Furthermore, sodium nitroprusside can enhance K^+ current in rabbit isolated pulmonary arterial smooth-muscle cells (Clapp & Gurney 1989), and cGMP can increase the activity of Ca^{2+}-activated K^+ channels in membrane patches from vascular smooth muscle when it is applied to the cytoplasmic side (Williams et al 1988). The authors of the latter study suggested that GMP may be the physiological activator of the channel, rather than cGMP, because it was more effective. Thus, in some but not all blood vessels, nitrovasodilators may act in a way comparable with the K^+-channel openers, causing an increased K^+ efflux from the smooth muscle cells, leading to membrane hyperpolarization and muscle relaxation. Nevertheless, hyperpolarization is unlikely to be a major contributor to vasodilation, because vasodilation occurs without hyperpolarization in several blood vessels (Ito et al 1980), and non-selective K^+-channel-blocking drugs have little effect on the relaxation induced by sodium nitroprusside, ANP or NO in rabbit pulmonary artery (Foley, Pitt, Davey & Gurney, unpublished observations), where sodium nitroprusside has been shown to cause hyperpolarization (Ito et al 1978). The anti-anginal drug nicorandil behaves both as a nitrovasodilator and a powerful K^+-channel opener. It is an organic nitrate ester that elevates intracellular cGMP levels (Endoh & Taira 1983). Although cGMP may contribute to its membrane hyperpolarizing effect, K^+-channel opening appears to be largely a direct action, independent of cGMP (Kajioka et al 1990).

Since nitrovasodilators inhibit agonist-induced contractions more effectively than depolarization-induced contractions, the prime target for these drugs must be a pathway that is selectively activated in the presence of an agonist. Vasoconstrictors elevate the intracellular Ca^{2+} concentration, independently of membrane depolarization, through receptor-activated Ca^{2+} influx and IP_3-mediated Ca^{2+} release from the sarcoplasmic reticulum. There is evidence that both of these pathways are modulated by nitrovasodilators. For example, the agonist-induced Ca^{2+} influx is more effectively blocked by sodium nitroprusside than the depolarization-induced Ca^{2+} influx (Karaki et al 1988; Magliola & Jones 1990). One of the proteins phosphorylated by G-kinase has been identified as phospholamban (Sarcevic et al 1989), an endogenous regulator of the sarcoplasmic reticulum Ca^{2+}, Mg^{2+}-ATPase. Enhanced uptake of Ca^{2+} into the sarcoplasmic reticulum by cGMP is further supported by electrophy-

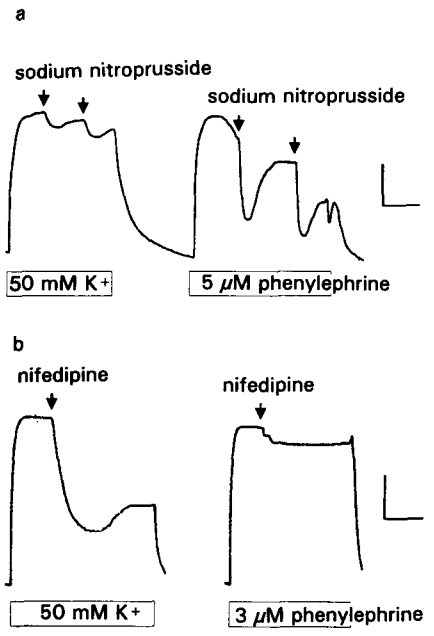

FIG. 4. Comparison of the effects of sodium nitroprusside and nifedipine on agonist-induced and K^+-induced contractions. The tension developed by an isolated strip of rabbit main pulmonary artery was measured and (a) sodium nitroprusside (0·1 μM) or (b) nifedipine (0·5 μM) perfused across the tissue for 30 s, in the presence of either 50 mM K^+ or phenylephrine. The relaxant effect of sodium nitroprusside was greater in the presence of phenylephrine, whereas the response to nifedipine showed the opposite trend. Calibration bars correspond to 10 min and 350 mg.

siological (Komori & Bolton 1989; Clapp & Gurney 1991b) and Ca^{2+} flux experiments (Twort & Van Breemen 1988). In addition, cGMP may inhibit the agonist-induced release of Ca^{2+} from the sarcoplasmic reticulum, by inhibiting phospholipase C and the production of IP_3 (Hirata et al 1990).

Several additional mechanisms have been proposed to explain cGMP-dependent vasodilation. These include a reduction in the sensitivity of the contractile proteins to Ca^{2+} (Nishimura & Van Breemen 1989), an action that may be linked to cGMP-dependent phosphorylation of myosin light-chain kinase (Nishikawa et al 1984), and stimulation of the plasmalemmal Ca^{2+}, Mg^{2+}-ATPase, which pumps Ca^{2+} out of the smooth muscle cell (Rashatwar et al 1987; Baltensperger et al 1990). Thus there are multiple sites of action in the smooth muscle cell through which cGMP may cause relaxation. It is not yet understood which of these mechanisms is primarily responsible for NO-induced vasodilation in-vivo.

Hydralazine

Hydralazine cannot clearly be classified with any of the types of vasodilator discussed above. Most pharmacology text books describe it as a vasodilator with unknown mechanism. Even though hydralazine has been in clinical use for over 40 years, remarkably little is known about how it acts to produce vasodilation. It mainly affects the arteries and arterioles, having little effect on the venous system. Consequently it causes a fall in blood pressure associated with reflex tachycardia and increased cardiac output. Although it is an effective antihypertensive, its use is limited by a variety of unacceptable side-effects and the dependence of its metabolism on the acetylator status of an individual. It is normally used in combination with other drugs.

Hydralazine acts directly on arterial smooth muscle, although a part of its effect may require the presence of an intact endothelium (Kreye 1984; Yen et al 1989). Hydralazine has been demonstrated to release NO in-vitro (Kruszyna et al 1987), suggesting that it might act like nitroprusside or the organic nitrates. On the other hand, the lack of effect of hydralazine on venous smooth muscle argues strongly against this. Furthermore, hydralazine does not elevate smooth muscle cGMP levels, and its vasorelaxant action is not prevented by methylene blue, an inhibitor of guanylyl cyclase (Yen et al 1989). Hydralazine has been found to hyperpolarize isolated arteries (Kreye 1984), so it may act, in part, like a K^+-channel opener. Consistent with this idea, hydralazine inhibits arterial contractions brought about by moderate elevation of the extracellular K^+ concentration (Barron et al 1977; Cook et al 1988), but it is less effective at higher K^+ concentrations (Khayyal et al 1981). As noted above, this is a property characteristic of K^+-channel openers (Fig. 3). The lack of effect of hydralazine at high extracellular K^+ concentrations rules out Ca^{2+} antagonism as a significant mechanism of action, since Ca^{2+} antagonists are most potent under these conditions (Fig. 2). Hydralazine does, however, inhibit Ca^{2+} influx (McLean et al 1978a). Since it is more effective at relaxing noradrenaline-induced contractures than K^+-induced contractures (McLean et al 1978b), inhibition of receptor-activated Ca^{2+} influx is a possible mechanism. Finally, hydralazine inhibits noradrenaline-induced contractions in the absence of extracellular Ca^{2+} (Orallo et al 1991), implying that it interferes with Ca^{2+} mobilization from intracellular stores. Thus progress has been made in understanding how hydralazine produces vasodilation, although the details of its various actions have yet to be determined.

Summary and Future Directions

Vasodilation can be accomplished by interfering with Ca^{2+} mobilization in vascular smooth muscle cells, or by reducing the sensitivity of the contractile proteins to Ca^{2+}. The currently available vasodilators act principally by lowering intracellular Ca^{2+} levels or preventing Ca^{2+} from rising in response to a contractile stimulus. However, the different types of vasodilator achieve this in different ways. The Ca^{2+} antagonists and K^+-channel openers have a specific target in the cell, namely the voltage-operated Ca^{2+} channel and an ATP-sensitive K^+ channel. Other drugs such as sodium nitroprusside, the organic nitrates and hydralazine are less selective, in that they influence multiple Ca^{2+}-generating or Ca^{2+}-removal pathways. Although several actions of these drugs have been identified at the cellular level, we still do not know which ones are responsible for producing vasodilation. Work is continuing in an attempt to establish the relative contribution that each action makes to the overall effects of the drugs. Knowledge of the principle mechanisms of action of a drug and its relative effects on different blood vessels, can be used to elucidate the principal pathways involved in the

normal generation and maintenance of tension in different blood vessels. Also, by studying the molecular mechanisms by which different vasodilators work, it is hoped that new potential targets for drug action might be discovered, and that eventually new drugs with different or improved selectivities might be developed. A drug that could selectively lower blood pressure, while causing minimal side effects, in a large proportion of the population would be a major advance in cardiovascular medicine.

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