Cardiovascular Drug Reviews
Vol. 10, No. 2, pp. 243–258

Mechanisms of Vasorelaxation

Anna F. Dominiczak and *David F. Bohr

*MRC Blood Pressure Unit, Western Infirmary, Glasgow, Scotland and *Department of Physiology, University of Michigan, Ann Arbor, Michigan, U.S.A.*

Key Words: Vasorelaxation—Vascular smooth muscle—Calcium—Cyclic nucleotides.

The regulation of vascular resistance is critical to the maintenance of circulatory homeostasis. The primary regulator of this resistance is the level of contraction of vascular smooth muscle, which is determined by the balance of factors that cause contraction with those that cause relaxation of this muscle. This review will feature the latter. However, to do this in a meaningful manner, specific contractile factors will also be considered.

Under physiological conditions, vascular smooth muscle (VSM) contraction requires a concentration of ionized calcium ($[Ca^{2+}]_i$) in the cytosol greater than 10^{-7} *M* and an energy source, ATP. Normally, $[Ca^{2+}]_i$ is the regulated variable that determines the magnitude of contraction (VSM tone). Relaxation is effected by mechanisms represented in Fig. 1 that reduce $[Ca^{2+}]_i$ and are therefore relevant to this review. Two of the mechanisms utilize active pumps requiring energy from ATP to move calcium out of the cytosol against large concentration gradients. These are the ATPase of the calcium efflux pump in the plasma membrane and the ATPase that sequesters calcium into the sarcoplasmic reticulum (SR). The third calcium-lowering mechanism is the sodium–calcium exchanger. This exchanger couples the energy derived from the movement of sodium down its concentration gradient into the cell to the extrusion of calcium from the cell.

The concentration of ionized calcium inside the cell is a net value determined by the relative rates of calcium movement into the cell and the sum of the activities of these three $[Ca^{2+}]_i$-lowering mechanisms. The nature of this balance indicates yet another means by which $[Ca^{2+}]_i$ may be reduced to produce VSM relaxation. This is obviously a reduction in the rate of calcium entry into the cytosol, as would result from the action of a calcium channel antagonist, or, indirectly, by hyperpolarization of the plasma membrane or interference with other calcium delivery systems.

CYCLIC NUCLEOTIDES

Cyclic nucleotides are second messengers that play an important role in regulating $[Ca^{2+}]_i$. They accomplish this regulation through their influence on the mechanisms listed

Address correspondence and reprint requests to Dr. A. F. Dominiczak at MRC Blood Pressure Unit, Western Infirmary, Glasgow, Scotland G11, 6NT.

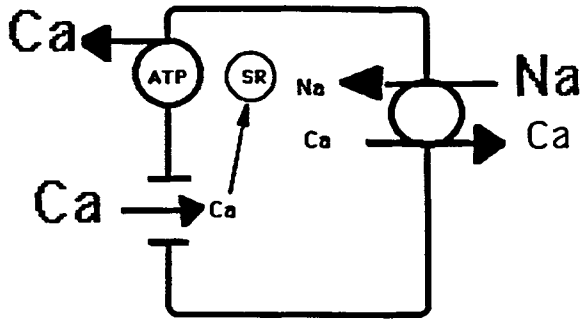

FIG. 1. Mechanisms by which cytosolic $[Ca^{2+}]_i$ is lowered to produce vascular smooth muscle relaxation include extrusion through the plasma membrane, sequestration into the sarcoplasmic reticulum (SR), and the sodium/calcium exchanger. Cytosolic $[Ca^{2+}]_i$ can also be lowered by decreasing the rate of entry of calcium using calcium channel antagonists or potassium channel openers. The latter produce plasma membrane hyperpolarization, closing voltage-sensitive calcium channels.

in the preceding paragraphs. Their importance in this and other regulatory roles was recognized by the award of the Nobel Prize in 1971 to Dr. Earl Sutherland for the discovery of cyclic adenosine monophosphate (cAMP) in 1957 (62).

These second messengers are remarkably diverse in their regulation of cellular functions, yet all of their actions are mediated by their activation of protein kinases. These are enzymes that transfer a phosphate group from ATP to another protein. This phosphorylation changes the activity of the second protein, causing the cellular response (secretion, contraction, or lowering of $[Ca^{2+}]_i$ depending on the specialized function of the protein that was phosphorylated).

The first of these cyclic nucleotides to be studied extensively was cAMP. This cyclic nucleotide is produced intracellularly from ATP by the activated enzyme adenylate cyclase. Activation of this enzyme, therefore, becomes the important means by which this second messenger is produced. Of particular relevance to the regulation of VSM is the fact that β-adrenergic receptors of this muscle activate adenylate cyclase. Catecholamines that bind to these receptors cause an accumulation of cAMP in the cell, followed by relaxation of VSM. Adenosine, arising from metabolic activity of the parenchyma, is an important paracrine messenger that also causes VSM relaxation by activating adenylate cyclase to produce cAMP (42). Interesting observations have been made bearing on the possible mechanism by which cAMP causes this relaxation.

One of these mechanisms involves a deactivation of the basic process by which the contractile proteins cause contraction. Rembold and Murphy (56) established that myosin light chain (MLC) (a 20 kDa protein) phosphorylation is necessary and sufficient for contraction of this smooth muscle. It follows that dephosphorylation would inevitably lead to relaxation. This phosphorylation is caused by the calcium–calmodulin-dependent enzyme, myosin light chain kinase (MLCK). This enzyme in turn can be phosphorylated by a cyclic AMP-dependent kinase and when MLCK is phosphorylated, its activity is decreased. Adelstein has presented extensive evidence (2) supporting the hypothesis that cAMP causes relaxation by this deactivation of MLCK with the resultant decreased phosphorylation of MLC.

Gerthoffer et al. (24) presented evidence indicating that although cAMP may facilitate

relaxation by dephosphorylating MLC, this is not the primary mechanism by which cAMP produces relaxation. They studied relaxation of phenylephrine-contracted VSM following rinsing of phenylephrine from the bath. The relaxation was normally slow, being incomplete 15 min after the bath had been rinsed. Yet they had determined that MLC was completely dephosphorylated 2 min after the rinse, corresponding to the time when $[Ca^{2+}]_i$ had returned to the resting level. Adding forskolin (an activator of adenylate cyclase) to the bath during the slow relaxation following this 2 min period caused a great increase in the rate of relaxation (Fig. 2). They argue that the elevated levels of cAMP caused by the addition of forskolin could not have caused this relaxation by phosphorylating and inactivating MLCK because the light chain was already completely dephosphorylated. Cyclic AMP must act through another mechanism to produce this relaxation. They suggest that this relaxation results from the activation of another cAMP-dependent kinase that activates a substrate protein capable of lowering the residual slightly elevated $[Ca^{2+}]_i$ responsible for delaying the relaxation. This phosphorylated substrate could be any one of the three calcium handling systems illustrated in Fig. 1 that are capable of lowering $[Ca^{2+}]_i$. Alternatively, the substrate could be a potassium channel protein, the activation of which would result in hyperpolarization, thereby decreasing calcium influx.

Yet another mechanism of relaxation by cyclic nucleotides has recently received supporting evidence. This is an inactivation of the sequence of events whereby a vasoconstrictor agonist causes an increase in cytosolic calcium. When the agonist binds to its specific membrane receptor, the receptor activates a G protein in the membrane that in turn activates the enzyme phospholipase C, which cleaves phosphoinositide into inositide trisphosphate (IP_3) and diacylglycerol. IP_3 escapes into the cytosol and causes the release of calcium from the SR. Rapoport et al. (53) reported that the inhibition by cyclic nucleotide of a norepinephrine (NE) -induced contraction of VSM was accompanied by a decrease in inositide phosphate production. Hirata et al. (28) elaborated on this observation, demonstrating that the inhibition resulted from a phosphorylation that inactivated the

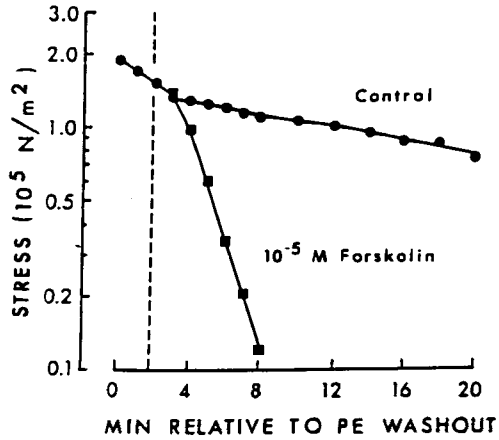

FIG. 2. The effect of forskolin (activator of adenylate cyclase) on relaxation after phenyleprine (PE) washout. Control relaxations (n = 4) were obtained after 15-min contractions with PE. Forskolin was added (dashed line) 2 min after PE washout at a time when the myosin light chain was already dephosphorylated. Reprinted from ref. 24 with permission.

G protein so that phospholipase C was no longer activated. Cyclic guanosine monophosphate (cGMP) was far more active than cAMP in producing this inhibition.

An ingenious study by Lincoln et al. (38) has recently shifted the focus of the mechanism of action of forskolin from cAMP to cGMP. These investigators monitored the increased $[Ca^{2+}]_i$ caused by arginine–vasopressin (AVP) in cultured rat aorta cells. They observed that in cells from primary cultures, this response was depressed following activation of adenylate cyclase with forskolin or with a β-adrenergic agonist. However, they also observed that if this study were carried out on cells after multiple passages, forskolin potentiated the increase in $[Ca^{2+}]_i$ caused by AVP. They hypothesized that this shift in response to forskolin was brought about by the loss of cGMP-dependent protein kinase with multiple passages. They tested this hypothesis by reloading these cells with cGMP-dependent protein kinase (using a technique of osmolarity changes to get the kinase into the cell). Once the cells were reloaded, they regained their depressant response to forskolin. They suggested that cAMP can activate cGMP-dependent kinase and present the sequence shown in Fig. 3. They conclude that "cAMP can produce both increases in Ca^{2+} levels and decreases in Ca^{2+} levels, depending on the absence or presence of cGMP-dependent protein kinase in the cell."

Francis et al. (21) came to a similar conclusion regarding the importance of cGMP from a study of many analogues of cAMP and cGMP. They compared the ability of each of the analogues to produce relaxation of the pig coronary artery to its ability to activate purified cGMP-dependent or cAMP-dependent protein kinases. The very close correlation between relaxation and activation of cGMP-dependent protein kinase supported a role for cGMP kinase but not for cAMP kinase in decreasing smooth muscle contraction.

The discovery and characterization of cGMP followed that of cAMP by about one decade. In 1963, it was discovered in rat urine (3) and its role in smooth muscle relaxation was identified in 1977 (32). By 1986, when Murad (43) reviewed the subject, it was recognized as the most important second messenger involved in VSM relaxation. Both the endothelium-derived relaxing factor (EDRF) and the atrial natriuretic factor (ANF) were

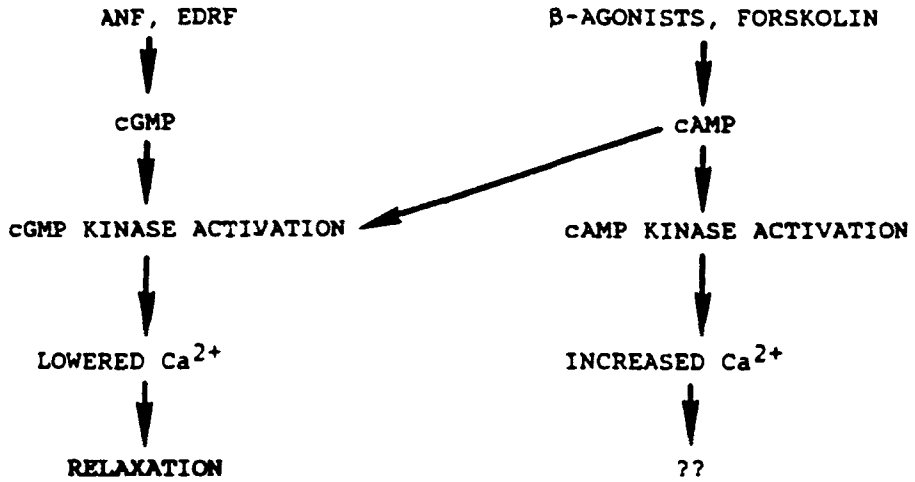

FIG. 3. Model for activation of cGMP-dependent protein kinase by cAMP, ANF, atrial natriuretic factor; EDRF, endothelium-derived relaxing factor. Reprinted from ref. 38 with permission.

found to produce relaxation by activation of guanylate cyclase and the production of cGMP. Pharmacologically, a large number of agents, the "nitrovasodilators," were found to produce their effects by the formation of reactive nitric oxide, which increased cGMP synthesis.

The increased production of cGMP initiated by these physiological and pharmacological stimulators of guanylate cyclase is accompanied by a decrease in $[Ca^{2+}]_i$. Without knowing the exact mechanism of this action of cGMP, it is safe to assume that the decrease in $[Ca^{2+}]_i$ results from the action of a cGMP-dependent protein kinase. It is not known which protein is phosphorylated by this enzyme, and the details of the mechanism by which cellular function is altered to cause a decrease in $[Ca^{2+}]_i$ remain undefined. Characterization of this cellular function is the information most needed to understand the mechanism of VSM relaxation.

HYPOXIC VASODILATION

Hypoxic vasodilation is an important condition responsible for VSM relaxation in both the physiological regulation and pathophysiological effects on blood flow. Physiologically, hypoxia develops when the blood flow to an organ fails to meet the energy requirements of the organ. The resultant hypoxic vasodilation increases blood flow, tending to correct the imbalance. Pathophysiologically, this type of VSM relaxation occurs with abnormal deprivation of flow, such as in coronary or cerebral artery occlusion.

Adenosine triphosphate (ATP) provides the energy required for chemomechanical transduction by the contractile proteins. It seems reasonable to expect that hypoxic vasodilation results from an inadequate aerobic production of this energy source. Experimental observations have established that the mechanism is not this simple.

The chemomechanical transduction, measured by the velocity of isotonic shortening, falls to 50% in smooth muscle when the ATP concentration is reduced to 0.1 m*M* (27). This observation is in accord with the findings of Moreland et al. (41) that force development by the nonphysiological stimulus, 50 m*M* KCl, was reduced to 50% when the ATP concentration had fallen to 0.1 m*M*. When, instead of KCl, the contraction was caused by the physiological agonist norepinephrine (NE), the force had already fallen to 50% when the ATP concentration was still at the higher level of 0.58 m*M*. These observations lead to two conclusions: (a) mechanisms of hypoxic relaxation differ depending on whether the contraction is caused by KCl or by NE, and (b) the rate-limiting mechanism for a hypoxic relaxation of NE-induced contraction of VSM cannot be the actomyosin ATPase activity of the chemomechanical transducer, because, as noted above, actomyosin ATPase operates at a half-maximum level when the ATP concentration is much lower, i.e., 0.1 m*M*.

Moreland et al. (41) evaluated other mechanisms that may be responsible for the VSM relaxation caused by hypoxia. They presented these in their model (Fig. 4) that depicts other energy-requiring processes, possibly responsible for hypoxic vasodilation.

Energy in the form of ATP is required for the active removal of Ca^{2+} from the cytosol. However, curtailing by hypoxia the activities of these pumps that extrude or sequester Ca^{2+} would increase rather than decrease $[Ca^{2+}]$. Hypoxic vasodilation would not be expected from this change.

The pump that moves Na^+ out of the cell and K^+ into the cell against concentration

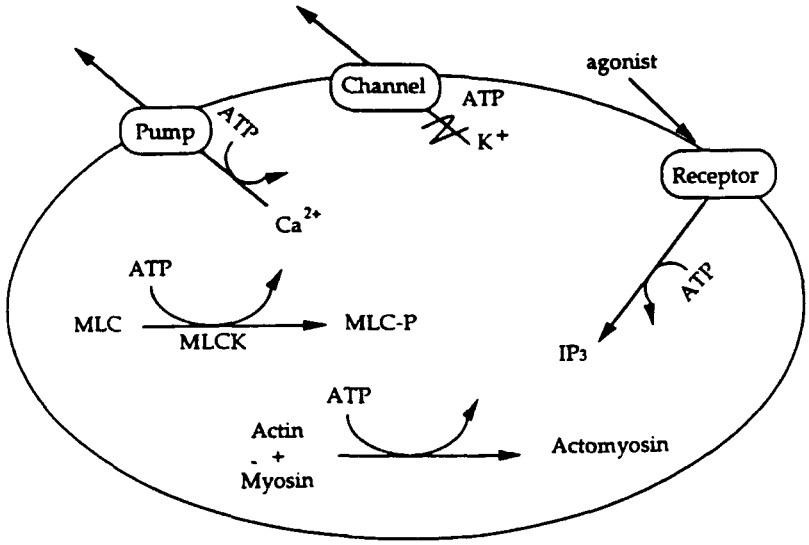

FIG. 4. Model of vascular smooth muscle cell showing possible sites of energy-dependent reactions affected by hypoxia: calcium extrusion pump, ATP-dependent K^+ channel, inositol trisphosphate (IP_3) cascade, myosin light chain kinase phosphorylation, actomyosin ATPase, and Na^+,K^+–ATPase (not shown). Reprinted from ref. 41 with permission.

gradients is also driven by energy derived from the hydrolysis of ATP. Since this Na^+, K^+–ATPase moves more K^+ out of the cell than Na^+ into the cell, it is electrogenic, causing membrane hyperpolarization, capable of producing VSM relaxation (11). A decrease in ATP concentration resulting from hypoxia would be expected to decrease pump activity, which would depolarize the membrane, making it more excitable. This effect could not be responsible for VSM relaxation. This action would be comparable to that of the physiologically occurring pump inhibitor, ouabain (26). This factor increases vascular reactivity and is a putative factor in the mechanism of hypertension.

Another membrane transport system to be considered in this context is the ATP-dependent potassium channel. Potassium efflux through this channel causes hyperpolarization and, therefore, VSM relaxation. Normally, ATP inhibits this channel. The hypoxic lowering of ATP concentration would be expected to diminish the inhibition, permitting the channel activity to increase, resulting in hyperpolarization and VSM relaxation. The problem with this putative mechanism is that, whereas 50% hypoxic relaxation of contractions caused by NE or KCl occur at ATP concentrations of 0.58 and 0.1 m*M*, respectively, the channel is maximally inhibited by much lower ATPase concentrations (16). This evidence against the involvement of an ATP-dependent K channel is supported by the observation that glyburide, which blocks this channel does not alter hypoxic relaxation of VSM (41).

The inositol phosphate cascade must also be considered as a site of action of hypoxia. The contractile response to NE utilizes this cascade, whereas that to KCl does not. The involvement of this system is suggested by the observation that the contractile response to NE is more sensitive to hypoxia than is that to KCl (41). The inositol phosphate cascade

requires much energy from ATP (41) and its activity is altered in hypoxia. The sensitivity of the NE response to hypoxia probably reflects a failure of the inositol phosphate cascade in its release of calcium from the SR.

In situ, paracrine influences arising from the parenchyma play an important role in hypoxic vasodilation. Hyperosmolarity, or increased potassium or CO_2 concentrations coming from the parenchymal metabolism, may contribute to this vasodilation. Adenosine is the parenchymal product that has received the most attention for this paracrine vasodilator role. It acts on the endothelial cell to release EDRF, which causes VSM relaxation by activating guanylate cyclase and thereby producing cGMP (42).

ENDOTHELIUM

An Endothelium-Derived Relaxing Factor—Nitric Oxide

Furchgott and Zawadzki (23) discovered that stimulation of muscarinic receptors on endothelial cells triggered the release of a substance that relaxed the underlying VSM. This EDRF relaxes VSM by elevating cGMP levels. EDRF has been shown to stimulate soluble guanylate cyclase, and the possible identity of EDRF and nitric oxide was established (30,49). The actions of both EDRF and nitric oxide are inhibited by hemoglobin and potentiated by superoxide dismutase. The ultimate proof that EDRF is identical to nitric oxide was obtained by Palmer et al. (49). They showed that nitric oxide was released from endothelial cells by bradykinin in amounts that accounted for the actions of EDRF. Nitric oxide is produced from arginine and its formation can be blocked by the arginine analogue N^G-monomethyl-L-arginine. This effect can be overcome by an excess of L-arginine (48,54). The endothelial cells have mechanisms for maintaining their intracellular concentration of arginine, including intracellular generation from L-citrulline.

The intravenous infusion of N^G-monomethyl-L-arginine into rabbits, rats, or guinea pigs results in a prompt rise in blood pressure that can be reversed by L-arginine (55). These experiments suggest that the basal, continuous release of nitric oxide from the endothelial cells is necessary to keep the vessels in a dilated state. The same seems to be true regarding human resistance vessels because intra-arterial injection of N^G-monomethyl-L-arginine into the forearm causes prolonged vasoconstriction, reversible by L-arginine (67). These data led to the hypothesis that a deficiency of EDRF–nitric oxide release may contribute to the pathogenesis of hypertension (50,68). Data from several laboratories have examined endothelium-dependent relaxation in animal models of hypertension (35,39,40). Panza et al. (50) reported that endothelium-mediated vasodilation was impaired in patients with essential hypertension. It is possible that dysfunction of endothelial cells leading to decreased endothelium-dependent relaxation and/or increased endothelium-dependent contraction is present in human hypertension. This may contribute to increased total peripheral resistance and may either exacerbate or causally contribute to the hypertensive process.

EDRF–nitric oxide not only relaxes VSM cells, but also inhibits the aggregation and adhesion of platelets by increasing intraplatelet concentrations of cGMP (52). This effect of EDRF is similar to the effect of prostacyclin, and a synergism between EDRF–nitric oxide and prostacyclin in preventing platelet aggregation has been described (52).

Endothelium-Derived Hyperpolarization Factor—EDHF

Treatment of dog coronary artery with acetylcholine causes both relaxation and hyperpolarization of the VSM if the endothelium is intact (19). Neither occurs if the artery is denuded of its endothelium. The hyperpolarization of the VSM cell membrane results from an increase in potassium conductance. The action of the endothelium-derived hyperpolarizing factor (EDHF) released from the endothelium can be differentiated from that of EDRF and nitric oxide because the hyperpolarization is much more transient than the vascular relaxation. Furthermore, hyperpolarization persists after the effects of EDRF have been prevented by treatment with methylene blue or hemoglobin (17,34). The nature of EDHF is not known. Although the cyclo-oxygenase pathway does not seem to be involved in its production since indomethacin does not affect its production (19), inhibitors of the P450-dependent metabolism of arachidonic acid prevent the transient hyperpolarization, suggesting that EDHF may be a labile metabolite of arachidonic acid (59).

Prostacyclin

Prostacyclin is a major prostanoid produced by the endothelial cells. Apart from its role as an inhibitor of platelet aggregation, prostacyclin is also a powerful vasodilator. Both effects of prostacyclin are mediated through activation of adenylate cyclase, which leads to an increase in intracellular cAMP (63). Prostacyclin is broken down rapidly in plasma to the biologically inactive compound, 6-ketoprostaglandin $F_{1\alpha}$. Prostacyclin is produced in the endothelial cells in response to pulsatile pressure or stimuli such as bradykinin, thrombin, serotonin, and adenine nucleotides (10,68). Prostacyclin production is initiated by phospholipase A_2, which releases arachidonic acid from membrane phospholipids. The enzyme cyclo-oxygenase converts arachidonic acid into prostacyclin endoperoxides, and prostacyclin synthase finally forms prostacyclin from prostaglandin H_2.

Similarly to EDRF–nitric oxide, prostacyclin is a local (paracrine) hormone that is effective only in the immediate proximity of the cell that releases it. On the abluminal side of the vessel, prostacyclin causes relaxation of the surrounding smooth muscle, and in the lumen it prevents platelets from clumping onto the endothelium (68). The instability of prostacyclin in the circulation makes it difficult to administer therapeutically. Beneficial effects have been demonstrated in patients with peripheral vascular disease and with Raynaud's phenomenon who were given intravenous infusion of prostacyclin for several hours over a period of several days (20). A constant infusion of prostacyclin has also been used to treat primary pulmonary hypertension (31).

Figure 5 shows the interrelationship between prostacyclin and EDRF–nitric oxide. It should be stressed that the same chemical stimuli, or a change in membrane conformation induced by sheer stress, lead to the release of EDRF–nitric oxide and prostacyclin. For this reason, it has been suggested that these two substances are a common defense mechanism of the endothelium (ref. 68 and Fig. 5).

The important and complex role of products of the endothelium has recently been demonstrated in the regulation of corpus cavernosum smooth muscle tone. Relaxation of this vascular muscle allows for expansion of the lacunar spaces that compressed the outflow veins to cause an erection. Physiologically, this muscle is under the control of dilator nerves that mediate their action by acetylcholine and VIP. Recent in vitro obser-

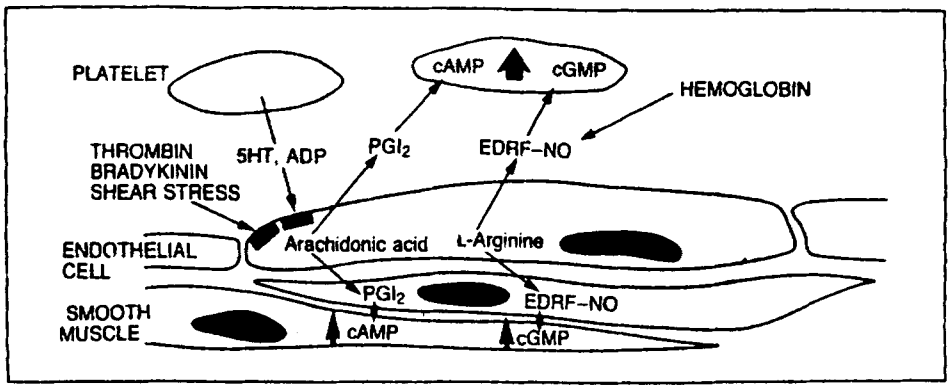

FIG. 5. Stimulation of receptors on the endothelial cells by serotonin (5-HT), adenosine diphosphate (ADP), thrombin, bradykinin, or sheer stress leads to the release of vasoactive mediators. Prostacyclin relaxes smooth muscle and inhibits platelet aggregation by increasing levels of cyclic adenosine monophosphate (cAMP). EDRF–nitric oxide also relaxes smooth muscle and inhibits platelet aggregation, but by increasing levels of cyclic guanosine monophosphate (cGMP). By increasing cAMP and cGMP simultaneously, prostacyclin and EDRF act synergistically. Modified from ref. 68 with permission.

vations characterized the regulation of rabbit and human corpus cavernosum smooth muscle (4). These studies demonstrated that relaxations of this muscle by acetylcholine, bradykinin, or substance P were endothelium dependent. The relaxation was accompanied by increased production of cGMP. It was attenuated by methylene blue (an inhibitor of guanylate cyclase) or by N^G-monomethyl-L-arginine. Interestingly, the relaxation was potentiated by indomethacin, which inhibited release from the endothelium of a cyclo-oxygenase product of arachidonic acid metabolism that caused contraction of the smooth muscle of the corpus cavernosum.

NEUROGENIC VASODILATION

The lengthy and controversial study of neurogenic vascular control can readily be accounted for by the complexity of this regulatory system. In the middle of the 19th century, Claude Bernard described his observation that cutting the rabbit's cervical sympathetic chain on one side caused the ear on that side to flush (7). He concluded that he had interrupted tonic sympathetic vasoconstrictor activity and thereby produced vasodilation. Six years later (8), he discovered vasodilator nerves when he stimulated the chorda tympany nerve and observed an increase in blood flow through the submaxillary gland. Early on, it was observed that many nerves contained both vasoconstrictor and vasodilator fibers. The effect of stimulating such a nerve gave an unpredictable result that depended on the relative numbers of each fiber being stimulated. In order to study either type of fiber, the effects of the other had to be blocked. Until the middle of this century, this was relatively simple since all neurogenic constriction was considered to be adrenergic, and all dilation was considered to be cholinergic. This concept changed, especially in the last decade when the number of established vasoactive, neurohumeral mediators increased exponentially. Figure 6 is a summation of the vasodilator mediators as depicted by Bevan and Brayden in 1987 (9). Not only is the number of mediators out of hand, but the

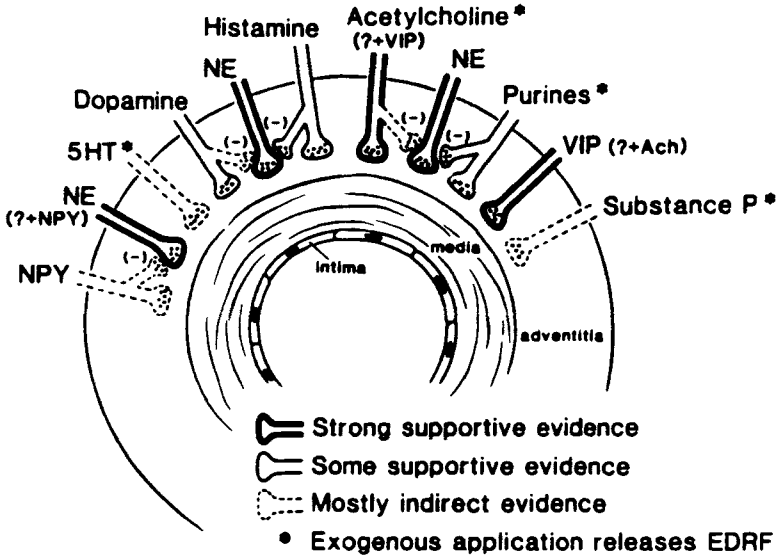

FIG. 6. Illustration from the 1987 review by Bevan and Brayden (9), illustrating putative neurotransmitters and possible sites of interactions between the different neuronal systems. In addition to the transmitters listed in this illustration, calcitonin gene-related peptide (cGRP) and nitric oxide (NO) are now established as vasodilators released from nerve terminals.

complexity is magnified by the presynaptic influences of several of the mediators on the activities of other vasoactive nerves. Rather than attempting to consider each of the many neurohumoral, vasodilator mediators, we refer the reader to recent reviews (9,46,47). We will merely describe four recently studied vasodilator systems as examples of this complexity.

NPY and CGRP

Kawasaki et al. (33) demonstrated convincingly that neuropeptide Y (NPY) modulates the release of calcitonin gene-related peptide (CGRP) from CGRP-containing vasodilator nerves in rat mesenteric arteries. The mesenteric arterial bed was perfused at a constant rate with physiological salt solution (PSS) containing methoxamine, to produce an active vascular tone, and guanethidine was used to abolish neurogenic adrenergic constriction. Under these conditions, stimulation of the nerve supply to the artery produced a vasodilation that was caused by CGRP release. Treating the preparation with exogenous NPY prevented this vasodilation and prevented the release of CGRP. NPY treatment did not prevent vasodilation caused by exogenously administered CGRP. Immunoreactive histochemical examination demonstrated that NPY and CGRP are present in separate nerves in the adventitia. The proximity of these nerves would permit the release of CGRP to be regulated by NPY released from its nerves.

Antidromic Vasodilation—Substance P

It has been known for well over a century that stimulation of the distal end of a cut sensory nerve causes arteriolar dilation. Immunocytochemical studies are consistent with

the conclusion that substance P is synthesized in sensory ganglia and is distributed to nerve terminals by rapid axonal transport (12). Substance P is one of the most potent vasodilator compounds known, acting primarily to release EDRF. Intradermal injection of substance P produces a dermal flare (25). This effect of exogenous substance P, as well as antidromic neurogenic vasodilation, is blocked by substance P antagonists (58,66).

Purinergic Nerves and ATP

Burnstock and his associates (15) have presented extensive evidence indicating that ATP is released by itself or is coreleased with other mediators from nerves in the vascular adventitia. The potential complexity of this regulatory system was evident when we observed (18) that ATP could cause either contraction or relaxation when it acted directly on VSM. In addition, acting on the endothelial cell, it released both EDRF and an endothelium-derived contractile factor (EDCF), which included prostaglandins I_2 and E_2 and thromboxane A_2.

Nitroxidergic Nerves

Toda and Okamura (64,65) observed that the temporal artery (dog or monkey), denuded of its endothelium, contracted in response to nerve stimulation. The contraction was blocked by phentolamine, indicating that they had stimulated an adrenergic nerve. It was potentiated by N^G-nitro-L-arginine (L-NNA), a nitric oxide synthesis inhibitor. When the arteries were blocked by phentolamine and contracted with prostaglandin $F_{2\alpha}$, nerve stimulation resulted in VSM relaxation, which they established was caused by nitric oxide release, demonstrating a nitroxidergic nerve.

PHARMACOLOGICAL AGENTS

Nitrovasodilators

Nitroglycerin and the organic nitrates are prodrugs that required conversion to an active intracellular moiety, nitric oxide (NO), that initiates VSM relaxation. Vasodilation of arteries and veins occurs when the enzyme guanylate cyclase is activated by NO, causing the conversion of guanosine triphosphate (GTP) to cGMP. This is the final pathway for vascular dilation caused by nitrovasodilators as well as by EDRF with NO as a common denominator (1,44). The initial step of denitration of the organic nitrates requires reduced thiol groups in the form of cysteine (44). During nitrate biotransformation, there is an obligatory role for the oxidation of SH donors, as the nitrate molecule is converted to nitric acid and then to NO. Continuous nitrate administration results in the loss of reduced SH groups in the cytoplasm and thus leads to nitrate tolerance (45). In contrast, nitroprusside directly forms NO without relying on reduced SH availability, tolerance is not found with this compound (45).

Needleman et al. (44,45) suggested that thiol donors such as N-acetylcysteine (NAC) or methionine may be effective in preventing or reversing this tolerance. A number of studies have shown that nitrate tolerance can be partially reversed by the addition of NAC or methionine (22,37) but other studies have produced negative results (29,51). The goals

of enhancing nitrate activity with respect to smooth muscle responses (e.g., angina or congestive heart failure) with a thiol donor hold promise for continuing clinical investigation (15).

Calcium Channel Antagonists

The calcium channel antagonists are a chemically heterogenous group of agents that inhibit transport of calcium ions into the VSM cells by selectively binding with vascular receptor sites of calcium channels. These receptor sites are associated with a major protein of the L class of voltage-gated channels (6). As a result of reduced calcium ion entry, VSM tone is reduced, producing arteriolar dilation and a reduction in total peripheral resistance. Using a variety of biochemical, immunologic, and pharmacologic techniques, it has been established that the "L" channel consists of five subunits (α_1, α_2, β_1, γ and δ). The receptor sites for calcium antagonists are within the structure of the α_1 subunit (60). Calcium antagonists exert their effects by binding to the calcium channel α_1 subunit, probably with the highest affinity when the channel is in the depolarized or "inactivated" state (5).

Several laboratories are currently attempting to identify the specific binding sites on the α_1 subunit (36). This should allow the development of drugs with high tissue specificity and favorable cardiac protective abilities.

Potassium Channel Openers

Selective opening of ATP-regulated K^+ channels leads to an outward potassium current causing membrane hyperpolarization, which closes voltage-operated calcium channels. The resultant fall in cytosolic Ca^{2+} reduces VSM contractile activity (57). Several chemically different compounds belong to the class of pharmacological agents referred to as K^+ channel openers. Two established vasodilators, minoxidil and diazoxide, produce part of their effect via the opening of vascular K^+ channels.

Other agents sharing this mechanism of action include cromakalim, lemakalim, pinacidil, and nicorandil. The latter compound is also a potent stimulator of cGMP formation (mechanism of action of nitrates). Patch–clamp studies confirmed the presence of ATP-sensitive K^+ channels in VSM cells isolated from rabbit mesenteric arteries (61). The kinetic pattern of the channel activity was found to be similar to that already described for ATP-sensitive K^+ channels in cardiac and skeletal muscle. The conductance in smooth muscle cells was found to be 135 pS, which differentiated the ATP-sensitive K^+ channels of VSM from these channels in cardiac and skeletal muscle (conductance of 40–90 pS). These K^+ channels mediated the relaxation of arterial smooth muscle by K^+ channel openers and these effects are inhibited by glibenclamide, a blocker of ATP-sensitive K^+ channels (57,61).

In normotensive animals, K^+ channel openers decrease the blood pressure in a dose-dependent manner. Similar results have been obtained in hypertensive animals (13,14). The effects of K^+ channel openers on regional blood flow and vascular resistance have been explored in a number of studies. In anesthetized and conscious animals, these drugs increase coronary blood flow. This coronary vasodilation tends to be more pronounced in the epicardial regions than in the subendocardial area. Vasodilation is weaker in the brain,

small intestine, kidney, and skeletal muscle. This regional blood flow profile is different from that of calcium channel agonists, which are potent coronary, cerebral, and skeletal muscle vasodilators. The dilatation by K^+ channel openers also differs from that of converting enzyme inhibitors, which preferentially dilate the renal and skeletal muscle vascular beds (14).

The potential cardiovascular applications of this class of drugs are in the treatment of hypertension and coronary artery disease. The side effects of this group of agents are common to all peripheral vasodilators and include tachycardia, stimulation of plasma renin activity, and sodium and water retention. These effects could be minimized if K^+ channel openers are administered together with drugs possessing complementary mechanisms of action such as angiotensin converting enzyme inhibitors, β-adrenoceptor antagonists, and/or diuretics.

SUMMARY

The mechanisms of vasorelaxation play an essential role in the regulation of blood pressure and in the distribution of blood flow. The ultimate determinant of vasorelaxation is a lowering of the concentration of ionized calcium $[Ca^{2+}]_i$ in the VSM cell. This review considered first the intracellular mechanisms involving cyclic nucleotides and hypoxia as causes of VSM relaxation. Most physiological and pharmacological mechanisms responsible for this relaxation are mediated by an excess production of cGMP. Important vasodilator influences arise from the endothelium and from nerve terminals in the adventitia. Although several vasoactive factors are released from the endothelium, nitric oxide is its most important product. Many different neurohumoral factors that cause relaxation of VSM are released in the adventitia. One of them appears to be nitric oxide, duplicating the mechanism of the vasorelaxation from the endothelium. These two regulatory systems also interact when the neurohumoral agent stimulates the endothelial cell to release its vasoactive factors. This interaction is exemplified by the release from cholinergic nerves of acetylcholine, which acts on the endothelium of the lacunar spaces to produce nitric oxide, causing vasorelaxation of the corpus cavernosum.

Important pharmacological vasorelaxants act through the cellular mechanisms that we have reviewed in connection with the physiological relaxations of VSM: nitrovasodilators stimulate the production of cGMP and calcium channel antagonists and potassium channel openers reduce the rate of calcium entry into the cell, permitting the $[Ca^{2+}]_i$ to be lowered by the physiological mechanisms for lowering the levels of cytosolic calcium (Fig. 1).

Acknowledgment: This review was supported by grants from the British Heart Foundation, grant number 91/96, and from the National Heart Lung Blood Institute grant number HL18575. We thank Mrs. Betty Bunnell for preparation of this manuscript.

REFERENCES

1. Abrams J. Interactions between organic nitrates and thiol groups. *Am J Med* 1991;91(suppl 3c):106–12.
2. Adelstein RS, Conti MA, Hathaway DR. Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3′:5′-monophosphate-dependent protein kinase. *J Biol Chem* 1978;253: 8347–50.
3. Ashman DF, Lipton R, Melicow MM, Price TD. Isolation of adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate from rat urine. *Biochem Biophys Res Commun* 1963;11:330–4.

4. Azadzoi KM, Kim N, Brown M, Goldstein I, Cohen R, Saenz De Tejada I. Endothelium-derived nitric oxide and cyclooxygenase products modulate corpus cavernosum smooth muscle tone. *J Urol* 1992;147: 220–5.
5. Bean BP. Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proc Natl Acad Sci USA* 1984;81:6388–92.
6. Bean BP. Pharmacology of calcium channels in cardiac muscle, vascular muscle, and neurons. *Am J Hypertens* 1991;4:393S–5.
7. Bernard C. Sur les éffets de la section de la portion encephalique du grand sympathique. *C R Soc Biol* 1852;40:168–9.
8. Bernard C. Sur une expérience relative a l'influence que les nerfs exercent sur les glandes, et particulierement aux phenomènes de circulation pendant la secrétion glandulaire. *C R Soc Biol* 1858;46:29–30.
9. Bevan JA, Brayden JE. Nonadrenergic neural vasodilator mechanisms. *Circ Res* 1987;60:309–26.
10. Bhagyalakshmi A, Frangos JA. Mechanism of sheer-induced prostacyclin production in endothelial cells. *Biochem Biophys Res Commun* 1989;158:31–7.
11. Bonaccorsi A, Hermsmeyer K, Aprigliano O, Smith CB, Bohr DF. Mechanism of potassium relaxation of arterial muscle. *Blood Vessels* 1977;14:261–76.
12. Brodin E, Gazelius B, Olgart L, Nilsson G. Tissue concentration and release of substance P-like immunoreactivity in the dental pulp. *Acta Physiol Scand* 1981;111:141–9.
13. Buckingham RE. Studies on the anti-vasoconstrictor activity of BRL 34915 in spontaneously hypertensive rats; a comparison with nifedipine. *Br J Pharmacol* 1988;93:541–52.
14. Buckingham RE, Clapham JC, Hamilton TC, Longman SD, Norton J, Poyser RH. BRL 34915, a novel antihypertensive agent: comparison of effects on blood pressure and other haemodynamic parameters with those of nifedipine in animal models. *J Cardiovasc Pharmacol* 1986;8:798–804.
15. Burnstock G, Kennedy C. A dual function for adenosine 5′-triphosphate in the regulation of vascular tone. *Circ Res* 1985;58:319–30.
16. Cameron JS, Kimura S, Jackson-Burns DA, Smith DB, Bassett AL. ATP-sensitive K^+ channels are altered in hypertrophied ventricular myocytes. *Am J Physiol* 1988;255:H1254–8.
17. Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br J Pharmacol* 1988;95:1165–74.
18. Dominiczak AF, Quilley J, Bohr DR. Contraction and relaxation of rat aorta in response to ATP. *Am J Physiol* 1991;261:H243–51.
19. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 1988;93:515–24.
20. Fiessinger JN, Schafer M. Trial of iloprost versus aspirin treatment for critical limb ischaemia of thromboangiitis obliterans. *Lancet* 1990;335:555–7.
21. Francis SH, Noblett BD, Todd BW, Wells JN, Corbin JD. Relaxation of vascular and tracheal smooth muscle by cyclic nucleotide analogs that preferentially activate purified cGMP-dependent protein kinase. *Mol Pharmacol* 1988;34:506–17.
22. Fung HL, Chong S, Kowaluk E, Hough K, Kakemi M. Mechanisms for the pharmacological interaction of organic nitrates with thiols. Existence of an extracellular pathway for the reversal of nitrate vascular tolerance by N-acetylcysteine. *J Pharmacol Exp Ther* 1989;245:524–30.
23. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond)* 1980;288:373–476.
24. Gerthoffer WT, Trevethick MA, Murphy RA. Myosin phophorylation and cyclic adenosine 3′,5′-monophosphate in relaxation of arterial smooth muscle by vasodilators. *Circ Res* 1984;54:83–9.
25. Hägermark Ö, Hökfelt T, Pernow B. Flare and itch induced by substance P in human skin. *J Invest Dermatol* 1978;71:233–5.
26. Hamlyn JM, Blaustein MP, Bove S, et al. Identification and characterization of an ouabain-like compound from human plasma. *Proc Natl Acad Sci USA* 1991;88:6259–63.
27. Hellstrand P, Arner A. Myosin light chain phosphorylation and cross-bridge cycle at low substrate concentration in chemically skinned guinea pig taenia coli. *Pflügers Arch* 1985;405:323–8.
28. Hirata M, Kohse KP, Chang CH, Ikebe T, Murad F. Mechanism of cyclic GMP inhibition of inositol phosphate formation in rat aorta segments and cultured bovine aortic smooth muscle cells. *J Biol Chem* 1990;265:1268–73.
29. Hogan JC, Lewis MJ, Henderson AH. N-acetylcysteine fails to attenuate hemodynamic tolerance to glyceryl trinitrate in healthy volunteers. *Br J Clin Pharmacol* 1989;28:421–6.
30. Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ Res* 1987;61:866–79.
31. Jones DK, Higenbottom TW, Wallwork J. Treatment of primary pulmonary hypertension with intravenous epoprostenol (prostacyclin). *Br Heart J* 1987;57:270–8.

32. Katsuki S, Arnold WP, Muirad F. Effects of sodium nitroprusside, nitroglycerin, and sodium azide on levels of cyclic nucleotides and mechanical activity of various tissues. *J Cyclic Nucleotide Res* 1977;3: 239–47.
33. Kawasaki H, Nuki C, Saito A, Takasaki K. NPY modulates neurotransmission of CGRP-containing vasodilator nerves in rat mesenteric arteries. *Am J Physiol* 1991;261:H683–90.
34. Komori K, Lorenz RR, Vanhoutte PM. Nictic oxide, ACh, and electrical and mechanical properties of canine arterial smooth muscle. *Am J Physiol* 1988;225:H207–12.
35. Konishi M, Sue C. Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. *Hypertension* 1983;5:881–6.
36. Koch WJ, Hui A, Shull GE, Ellinor P, Schwartz A. Characterization of cDNA clones encoding two putative isoforms of the α_1 subunit of the dihydropyridine-sensitive voltage-dependent calcium channel isolated from rat brain and rat aorta. *FEBS Lett* 1989;250:386–8.
37. Levy WS, Kutz RJ, Ruffalo RL, Leiboff RH, Wasserman AG. Potentiation of the hemodynamic effects of administered nitroglycerin by methionine. *Circulation* 1988;78:640–5.
38. Lincoln TM, Cornwell TL, Taylor AE. cGMP-dependent protein kinase mediates the reduction of Ca^{2+} by cAMP in vascular smooth muscle cells. *Am J Physiol* 1990;258:C399–407.
39. Lockette W, Otsuka Y, Carretero O. The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension* 1986;8(suppl II):II-61–6.
40. Luscher TF, Vanhoutte PM. Endothelium-dependent responses to platelets and serotonin in spontaneously hypertensive rats. *Hypertension* 1986;8(suppl II):II-55–60.
41. Moreland S, Coburn RF, Baron CB, Moreland RS. Mechanical and biochemical events during hypoxia-induced relaxations of rabbit aorta. *Adv Exp Biol Med* 1991;304:147–57.
42. Moritoki H, Matsugi T, Takase H, Ueda H, Tanioka A. Evidence for the involvement of cyclic GMP in adenosine-induced, age-dependent vasodilatation. *Br J Pharmacol* 1990;100:569–75.
43. Murad F. Cyclic guanosine monophosphate as a mediator of vasodilation. *J Clin Invest* 1986;78:1–5.
44. Needleman P. Biotransformation of organic nitrates. In: Needleman P, ed. *Organic nitrates*. New York: Springer-Verlag, 1975:57–95 (*Handbook of experimental pharmacology*, Vol. 40).
45. Needleman P, Johnson EM. The pharmacological and biochemical interaction of organic nitrates with sulphydryls: possible correlations with the mechanism for tolerance development, vasodilation and mitochondrial and enzyme reactions. In: Needleman P, ed. *Organic nitrates*. New York: Springer-Verlag, 1975:97–114. (*Handbook of experimental pharmacology*, Vol. 40).
46. Nobin A, Owman CH, Arneklo-Nobin B, eds. *Neuronal messengers in vascular function*. Amsterdam: Elsevier, 1987.
47. Owman C. Peptidergic vasodilator nerves in the peripheral circulation and in the vascular beds of the heart and brain. *Blood Vessels* 1990;27:73–93.
48. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* (*Lond*) 1988;333:664–6.
49. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* (*Lond*) 1987;327:524–6.
50. Panza JA, Quyyami AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22–7.
51. Parker JO, Farrell B, Lahey KA, Rose BF. Nitrate tolerance: the lack of the effect of N-acetylcysteine. *Circulation* 1987;76:572–6.
52. Radomski MW, Palmer RM, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987;92:639–6.
53. Rapoport RM, Schwartz K, Murad F. Effect of sodium–potassium pump inhibitors and membrane-depolarizing agents on sodium nitroprusside-induced relaxation and cyclic guanosine monophosphate accumulation in rat aorta. *Circ Res* 1985;57:64–170.
54. Rees DD, Palmer RM, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br J Pharmacol* 1989;96:418–24.
55. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989;86:3375–8.
56. Rembold CM, Murphy RA. Myoplasmic $[Ca^{2+}]$ determines myosin phosphorylation in agonist-stimulated swine arterial smooth muscle. *Circ Res* 1988;63:593–603.
57. Richer C, Pratz J, Mulder P, Mondot S, Giudicelli JF, Cavero I. Cardiovascular and biological effects of K^+ channel openers, a class of drugs with vasorelaxant and cardioprotective properties. *Life Sci* 1990;47: 1693–705.
58. Rosel S, Olgart L, Gazelius B, Panopoulos P, Folkers K, Hörig J. Inhibition of antidromic and substance P-induced vasodilatation by a substance P antagonist. *Acta Physiol Scand* 1981;111:381–2.

59. Rubanyi GM, McKinney M, Vanhoutte PM. Biphasic release of endothelium-derived relaxing factor(s) by acetylcholine from perfused canine femoral arteries. Characterization of muscarinic receptors. *J Pharmacol Exp Ther* 1987;240:802–8.
60. Schwartz A. Calcium antagonists: review and perspective on mechanism of action. *Am J Cardiol* 1989;64 (suppl I):3–7.
61. Standen NB, Quayle JM, Davies NW, Brayden JE, Hwang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 1989;245:177–80.
62. Sutherland EW, Rall TW. The properties of an adenine ribonucleotide produced with cellular particles, ATP, Mg^{++} and epinephrine or glucagon. *J Am Chem Soc* 1957;79:3608.
63. Tateson JE, Moncada S, Vane JR. Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins* 1977;13:389–97.
64. Toda N, Okamura T. Possible role of nitric oxide in transmitting information from vasodilator nerve to cerebroarterial muscle. *Biochem Biophys Res Commun* 1990;170:308–13.
65. Toda N, Okamura T. Reciprocal regulation by putatively nitroxidergic and adrenergic nerves of monkey and dog temporal arterial tone. *Am J Physiol* 1991;261:H1740–3.
66. Törnebrandt K, Nobin A, Owman CH. Contractile and dilatory action of neuropeptides on isolated human mesenteric blood vessels. *Peptides* 1987;8:251–6.
67. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989;2:997–1000.
68. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990;323:27–37.