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THE PHARMACOLOGICAL AND PHYSIOLOGICAL ROLE OF CYCLIC GMP IN VASCULAR SMOOTH MUSCLE RELAXATION

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INTRODUCTION

The biological role of cyclic GMP in vascular smooth muscle regulation is a relatively new concept that had its debut at a time when the biological significance of alterations in cellular levels of cyclic GMP was itself a controversial issue. Indeed, even today the physiological importance of this cyclic nucleotide remains unclear, although recent studies may be on the verge of providing important new insights. Information on the regulatory role of cyclic AMP, on the other hand, is far more prevalent, probably because such studies commenced about fifteen years prior to those on cyclic GMP, and many more investigators are presently studying cyclic AMP than are studying cyclic GMP.

Progress in cyclic GMP research seems to have been thwarted somewhat because certain concepts developed in the mid-1970s no longer appear to be tenable. Due in part to the paucity of basic information on cyclic GMP at the time, incompletely tested hypotheses were forwarded that suggested that cyclic GMP might be involved in the contraction of smooth muscle, including vascular smooth muscle. This was an attractive hypothesis because other studies had suggested that cyclic AMP was involved in smooth muscle relaxation, thereby providing the intriguing concept that by their opposing actions cyclic nucleotides could regulate smooth muscle function. However, surprising reports appeared illustrating that some clinically employed vasodilator drugs

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caused a marked accumulation of cyclic GMP, but not cyclic AMP, in vascular tissue. The initial message from these studies was that cyclic GMP might not be involved in the expression of smooth muscle contraction. Then a new insight into the significance of these early observations emerged, along with the question: could cyclic GMP possibly be involved in smooth muscle relaxation?

The objective of this review is to focus attention on the possibility that cyclic GMP is involved in vascular smooth muscle relaxation brought about not only pharmacologically but also by physiological means.

EARLY STUDIES ON CYCLIC GMP

The first reports on the role of cyclic GMP in smooth muscle function leaned toward the possibility that tissue cyclic GMP accumulation and contraction were closely related events. Several autacoids, muscarinic receptor agonists, and related calcium-dependent agents that caused smooth muscle contraction also caused cyclic GMP accumulation in these tissues (1–5). Both cellular responses were dependent on calcium, but contraction usually preceded any significant increase in tissue levels of cyclic GMP. Later, the subsequent accumulation of cyclic GMP was suggested to be the result of indirect actions of intracellular calcium on intracellular guanylate cyclase (6). In any event, this temporal relationship between contraction and cyclic GMP accumulation suggested that the latter event was a consequence rather than a cause of the former. Such a view was no longer consistent with the earlier concept that cyclic GMP was involved in smooth muscle contraction.

A further clue that cyclic GMP was not likely to be involved in contraction developed in 1975 when nitroglycerin, a potent vasodilator, was reported to elevate cyclic GMP levels in arterial and other tissues (7, 8). At about the same time, other reports appeared showing that sodium azide, hydroxylamine, and sodium nitrite activated soluble guanylate cylcase and stimulated tissue cyclic GMP accumulation (9–11). These chemical agents were well known to cause smooth muscle relaxation. Subsequently, sodium nitroprusside, nitroglycerin, and related smooth muscle relaxants were reported to increase muscle levels of cyclic GMP (12–14). The above studies were suggestive of the possibility that cyclic GMP might be involved in smooth muscle relaxation. However, other studies (15, 16) revealed that if this hypothesis were correct, it might not generally be applied to all smooth muscles. Thus, the interpretation of these data was unclear.

The first experiments in our laboratory on this subject were designed to test a relatively straightforward concept. Chemical agents known to activate soluble guanylate cyclase should cause vascular smooth muscle relaxation if cyclic GMP is involved in the relaxation process. In addition, since sodium nitroprusside is unstable in aqueous solution and releases nitric oxide and because nitric

oxide is a potent activator of guanylate cyclase, then nitric oxide itself should be a vasodilator. Moreover, unstable organic nitroso compounds, which release nitric oxide, should also cause relaxation. In our first series of experiments, nitric oxide gas and nitrosoguanidine compounds were demonstrated to cause potent and marked relaxation of precontracted helical strips of bovine coronary artery (17, 18). This was a novel observation that was extended to include other chemical agents related to nitric oxide (19–22). The concomitant observations that nitric oxide, nitroso compounds, and compounds capable of forming or releasing nitric oxide all activated soluble guanylate cyclase from vascular smooth muscle and elevated arterial levels of cyclic GMP but not cyclic AMP led us to believe that cyclic GMP is somehow involved in vascular smooth muscle relaxation elicited by pharmacological intervention (20, 21, 23, 24). Reports from other laboratories on the close relationship between arterial relaxation and cyclic GMP accumulation in the presence of standard vasodilators such as sodium nitroprusside and nitroglycerin soon followed (25–30). These observations indicated that vascular and nonvascular smooth muscle may differ in regard to the role of cyclic GMP. Tracheal smooth muscle, however, appears to respond similarly to vascular smooth muscle with respect to the effects of nitroso compounds that elevate tissue levels of cyclic GMP (13).

NITRIC OXIDE AS A VASCULAR SMOOTH MUSCLE RELAXANT

Keeping in mind the earlier observations inconsistent with a role for cyclic GMP in smooth muscle relaxation, several groups nevertheless made the decision to continue studies designed to test the hypothesis that cyclic GMP is involved in the relaxation of smooth muscle. In view of the clear indication that the most consistent data were obtained with known vasodilator drugs, we elected to restrict our studies to vascular smooth muscle. At this point, what was needed was a better understanding of the relationship between nitric oxide and certain vasodilators and a selective antagonist of this class of vascular smooth muscle relaxant.

An earlier suggestion that nitric oxide probably was the common factor involved in the activation of soluble guanylate cyclase by sodium nitroprusside, nitrites, organic nitrates, and nitroso compounds (9–11, 31–33) seemed very plausible. Moreover, the fact that nitric oxide is very lipophilic and unstable was consistent with the well-known transient hypotensive actions of the latter vasodilators. Thus, the attractive hypothesis that nitric oxide is responsible for the vascular effects of this class of vasodilator drug was set forth (17, 18, 20). Additional studies were consistent with this view. The direct release or formation of nitric oxide from sodium nitroprusside, sodium nitrite, amyl nitrite,

nitroglycerin, nitrosoguanidines, and related agents was demonstrated (34–39). The transient relaxant effects of nitric oxide in both arteries and veins were associated with equally transient increases in vascular cyclic GMP levels, whereas nitroglycerin elicited longer-lasting effects on both cellular events (20, 24). Nitroglycerin produces a much longer-lasting response, probably because once this highly lipophilic ester permeates the cell continual formation of nitric oxide occurs within the cell (21). The same appears true for sodium nitroprusside, nitroso compounds, other organic nitrate esters, and organic nitrite esters (21).

Several years ago, we suggested the use of the term *nitrogen oxide–containing vasodilators* for those hypotensive agents that possess, generate, or release nitric oxide (21). These drugs include inorganic and organic nitroso compounds and organic nitrite and nitrate esters. This terminology, or a related one, should be used instead of the older *direct-acting vasodilators* or *direct-acting spasmolytics* because much more information is now available on how these drugs work. It appears no longer necessary to employ the older and more ambiguous terminology.

METHYLENE BLUE AS A SELECTIVE ANTAGONIST

In an attempt to find antagonists of the relaxant effects of nitric oxide, the authors took advantage of previous reports that hemoglobin and methylene blue were inhibitors of guanylate cyclase activation by nitric oxide (40). Hemoglobin, myoglobin, related hemoproteins, and methylene blue were found to inhibit coronary arterial relaxation elicited by nitric oxide but not catecholamines (17–22). Hemoproteins, however, failed to inhibit the relaxant effects of sodium nitroprusside, organic nitrates, and nitrosoguanidines, whereas methylene blue was still an effective antagonist. At first, these observations were puzzling, but further experimentation cleared up the apparent enigma. High molecular–weight hemoproteins have a high affinity for nitric oxide and react with the latter to form relatively stable nitrosylhemoprotein complexes that cannot penetrate cells. Organic nitrates and nitrites and nitroso compounds are lipophilic substances and release or form nitric oxide within cells. Methylene blue is a vital biological stain that by definition permeates cell membranes. Therefore, hemoproteins cannot antagonize the effects of nitric oxide liberated intracellularly, whereas methylene blue can (17–22). Similarly, the highly charged inorganic species ferricyanide, which is an oxidant and inhibits guanylate cyclase activation by nitric oxide, as does methylene blue (40), cannot inhibit the relaxant effect of nitric oxide because the ferricyanide cannot enter the cell to interact with soluble guanylate cyclase (17). Since methylene blue inhibits vascular smooth muscle relaxation elicited by all of the nitric oxide–forming or nitric oxide–releasing vasodilator drugs without in-

fluencing relaxation elicited by other drugs such as isoproterenol, certain prostaglandins, and calcium antagonists (17, 19; L. J. Ignarro, P. J. Kadowitz, unpublished information), methylene blue is a very useful selective antagonist of the former class of vascular smooth muscle relaxant (19, 41). Chemically related vital biological stains (brilliant cresyl blue) are similarly effective.

Methylene blue inhibits not only the relaxant effect of the nitrogen oxide–containing vasodilators but also their stimulatory effects on vascular cyclic GMP accumulation (20, 21, 24). These observations are important for several reasons. First, these actions of methylene blue greatly strengthened our initial belief that cyclic GMP is involved in vascular smooth muscle relaxation. Second, these data illustrate the potential utility of methylene blue and related agents as pharmacological probes to discern other biological roles of cyclic GMP in cellular function. Third, the studies suggest strongly that the nitrogen oxide–containing vasodilators interact with intracellular receptors, the most apparent candidate being guanylate cyclase, to stimulate the formation of cyclic GMP and thereby generate the intracellular signal for initiating the relaxation process. More recent studies from this laboratory, based on the original observations by Craven & DeRubertis (33, 42), suggest that the intracellular receptor for nitric oxide is soluble guanylate cyclase-bound heme (43–45).

Methylene blue has also been employed to demonstrate unequivocally that cyclic GMP is not responsible for causing or promoting vascular smooth muscle contraction elicited by certain hormonal-like agents. Employing methylene blue as a pharmacological probe, Kukovetz and co-workers (46) showed that vascular smooth muscle contraction caused by acetylcholine is markedly enhanced by methylene blue and that this is associated with decreased tissue levels of cyclic GMP. Contrariwise, the cyclic GMP phosphodiesterase inhibitor M&B-22,948 elevates cyclic GMP levels and inhibits contractions caused by acetylcholine. By lowering resting tissue levels of cyclic GMP, methylene blue raises vascular smooth muscle tone (20, 21; L. J. Ignarro, P. J. Kadowitz, unpublished information). The cyclic GMP phosphodiesterase inhibitor, on the other hand, potentiates relaxation and cyclic GMP accumulation elicited by relaxants (28). These findings indicate clearly that cyclic GMP accumulation and vascular smooth muscle contraction can be completely dissociated. Moreover, the data further support the view that cyclic GMP accumulation and vascular smooth muscle relaxation are closely associated biological processes.

S-NITROSOTHIOLS AS ACTI

Discussions of the original observations on S-nitrosothiols as active intermediates and the rationale behind their design were reported previously (41). Briefly, thiols have been consistently found to enhance or unmask the activa-

tion of vascular soluble guanylate cyclase (21). Additional studies revealed that the nitrogen oxide–containing vasodilators react with thiols to generate chemically unstable S-nitrosothiols (21, 23, 38, 39). Organic nitrates reacted only with cysteine and formed S-nitrosocysteine, which is consistent with the observations that only cysteine enabled three different organic nitrates to activate purified soluble guanylate cyclase (21). A series of S-nitrosothiols were tested and found to elicit potent and marked relaxation of coronary artery, intrapulmonary artery and vein, and mesenteric artery (21, 22, 24). These observations, together with those that thiols react with nitrogen oxide–containing vasodilators to rapidly generate the corresponding S-nitrosothiols, suggested that S-nitrosothiols could serve as intracellular active intermediates of the parent drugs in the expression of vascular smooth muscle relaxation.

Subsequent experiments revealed that S-nitrosothiols caused a rapid accumulation of arterial and venous levels of cyclic GMP, which clearly preceded the onset of relaxation (21, 24). Moreover, both cellular responses were inhibited by methylene blue but not by propranolol, indomethacin, atropine, or antihistamines. The results of these in vitro experiments were confirmed in several in vivo studies. Intravenous injections of S-nitrosothiols in the anesthetized cat caused transient but marked decreases in systemic arterial pressure without appreciably altering cardiac output (21). Thus, a marked decrease in systemic vascular resistance had occurred. We have also shown that sodium nitroprusside, nitroglycerin, and S-nitrosothiols decrease pulmonary vascular resistance by dilating intrapulmonary veins and upstream segments when pulmonary vascular resistance is increased by an active process (47; H. L. Lippton, L. J. Ignarro, A. L. Hyman, P. J. Kadowitz, unpublished information). The latter observations are strikingly consistent with the clinical impressions and experimental findings (24) that veins are generally more sensitive than arteries to the relaxant and cyclic GMP accumulating effects of S-nitrosothiols, organic nitrates, and sodium nitroprusside. Thus, studies in the intact animal correlate closely with results obtained in isolated vessel segments, suggesting that isolated vessels provide a good model system with which to study relationships between vasodilatation and cyclic GMP accumulation. The hypotensive actions of these agents are unaltered by propranolol, atropine, or indomethacin. Additional in vivo studies showed that S-nitroso-N-acetylpenicillamine, nitroglycerin, and sodium nitroprusside produce almost identical qualitative hypotensive response in the feline mesenteric and intrapulmonary arterial vascular beds (H. L. Lippton, P. J. Kadowitz, L. J. Ignarro, unpublished information).

In all the in vivo experiments in which agents were injected systemically (iv), it was clear that the hemodynamic properties of the S-nitrosothiols are remarkably similar, if not identical, to those of sodium nitroprusside and nitroglycerin. All vasodilators displayed similar onset times of several seconds,

similar durations of action of 45–90 seconds, and little or no effect on cardiac output. That the half-lives of the S-nitrosothiols in oxygenated aqueous media are very short (21) is consistent with the transient effects not only of S-nitrosothiols but also of sodium nitroprusside and nitroglycerin on systemic arterial pressure.

On the basis of all of these observations, we offered the view that nitrogen oxide–containing vasodilators caused vascular smooth muscle relaxation by permeating smooth muscle cells and generating intracellular S-nitrosothiols, which rapidly decompose to liberate nitric oxide, resulting in the stimulation of cyclic GMP formation and consequent vascular smooth muscle relaxation (21). This hypothesis encompasses all of the observations that we and others have made during the past six years and is illustrated schematically in Figure 1.

THE MECHANISM OF TOLERANCE

Tolerance to the hypotensive action of organic nitrates in man is well known but little understood. Needleman and colleagues (48, 49) suggested earlier that vascular smooth muscle relaxation caused by organic nitrates may be dependent on free −SH groups in the tissue. Depletion of −SH groups by chemical agents, including nitroglycerin, caused a marked decrease in relaxation responses to organic nitrates but not to other vasodilators. Responsiveness was restored by treatment of vessels with sulfhydryl reducing agents. This earlier conclusion is consistent with more recent observations that organic nitrates may elicit vascular smooth muscle relaxation by first reacting with tissue −SH groups to generate highly active S-nitrosothiol intermediates that are responsible for the effects of the organic nitrates (21). Moreover, recent studies indicate clearly that arterial strips rendered tolerant to nitroglycerin also display equal tolerance to the arterial cyclic GMP accumulating effects of organic nitrates but not to these effects of sodium nitroprusside or cyclic GMP itself (29, 50, 51). Preliminary studies indicate that arterial and venous segments made tolerant to nitroglycerin are still fully responsive to S-nitrosothiols.

These observations suggest three things. First, the persistent close association between cyclic GMP accumulation and relaxation further supports the view that cyclic GMP is involved in vascular smooth muscle relaxation elicited through pharmacological intervention by nitrogen oxide–containing vasodilators. Second, organic nitrates are likely metabolized within vascular smooth muscle cells to S-nitrosothiols, which, because of their instability, liberate nitric oxide and stimulate cyclic GMP formation. Third, vascular tolerance to organic nitrates may be attributed to the loss of formation of S-nitrosothiols, thereby resulting in less cyclic GMP accumulation and decreased vascular smooth muscle relaxation.

A recent clinical study supports the above interpretations (52). Patients who

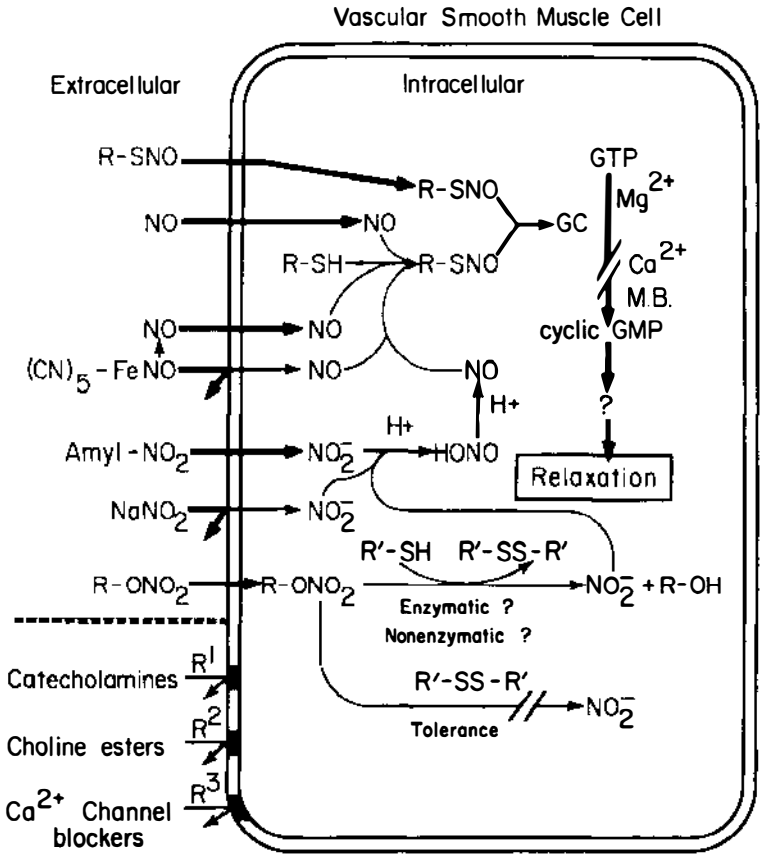

Figure 1 Schematic illustration of proposed mechanism of vascular smooth muscle relaxation produced by nitrogen oxide–containing vasodilators. Lipophilic substances permeate cells and form or release NO, either directly or through NO_2^-, which then reacts with thiol(s) to generate R-SNO. R-SNO, or NO released from R-SNO, activates GC to generate cyclic GMP. $NaNO_2$ is hydrophilic and is a very weak vasodilator. Nitroprusside (nitrosoferricyanide) is somewhat lipophilic, but is unstable in solution and liberates NO. Abbrevations: R-SNO, S-nitrosothiol; NO, nitric oxide; HONO, nitrous acid; $(CN)_5$-FeNO, nitroprusside; R-ONO_2, organic nitrate; R-OH, denitrated organic nitrate; R-SH low/high molecular weight thiol; R'-SH, thiol distinct from R-SH; GC, guanylate cyclase; M.B., methylene blue; R^1,R^2,R^3, extracellular receptors. [Reproduced with permission from (21).]

experienced varying degrees of tolerance to the hypotensive effects of nitroglycerin were given intravenous injections of N-acetylcysteine. The latter drug is a sulfhydryl reducing agent that can be administered systemically in relatively large doses without causing overt effects. Patients receiving N-acetylcysteine showed marked potentiation of the hypotensive effects of acutely administered nitroglycerin. These important clinical observations support

the views that tolerance to organic nitrates is due to tissue −SH depletion and that S-nitrosothiols are the active intracellular intermediates of organic nitrates.

THE MECHANISM OF VASODILATATION BY ACETYLCHOLINE

Until the recent discovery by Furchgott and co-workers (53) that arterial smooth muscle relaxation by acetylcholine is dependent on a functioning endothelial cell layer, acetylcholine was known to contract isolated vessels, although vasodilatation was the principal effect in vivo. Prior to this important advance, acetylcholine-elicited contractions were reported to be associated with a concomitant accumulation of tissue cyclic GMP (discussed above). These early observations prompted the premature conclusion that cyclic GMP was involved in the contractile process. Upon learning that acetylcholine can in fact relax vascular smooth muscle in vitro provided the endothelium remains intact, several groups of investigators began to elucidate the relationship between cyclic GMP and relaxation in response to acetylcholine and related vasodilators.

The first reports illustrated the close relationship between vascular smooth muscle relaxation and cyclic GMP accumulation elicited by acetylcholine on arterial segments possessing a functioning endothelium (54–57). Acetylcholine elicits concentration- and time-dependent increases in arterial cyclic GMP levels that correlate well with relaxation (54–57). A comprehensive time-course analysis revealed that cyclic GMP accumulation clearly precedes the onset of relaxation (57). Atropine, a muscarinic receptor antagonist, and methylene blue, a guanylate cyclase inhibitor, each abolished both cellular responses to acetylcholine (54, 57). Cyclic AMP levels were not altered by acetylcholine. Cyclic GMP phosphodiesterase inhibitors enhanced responses to acetylcholine (54).

The inhibition of acetylcholine-elicited cyclic GMP accumulation by atropine and methylene blue provides evidence that a link exists between muscarinic receptors and guanylate cyclase (57). As acetylcholine is well known not to directly activate guanylate cyclase, the stimulation of cyclic GMP formation inside vascular smooth muscle cells must be an indirect consequence of muscarinic receptor stimulation. The finding that endothelium-damaged arteries and endothelium-intact veins both contract and show small but significantly elevated cyclic GMP levels in response to acetylcholine indicates that vascular endothelium is not obligatory for stimulated cyclic GMP formation by acetylcholine (57). In bovine intrapulmonary arteries, the endothelium greatly enhances the capacity of acetylcholine to stimulate cyclic GMP formation, and this is accompanied by marked relaxation (57). Thus, arterial endothelial cells may generate one or more factors that either directly or indirectly activate

guanylate cyclase. This endothelium-derived factor(s) may be the same as or similar to that suggested for endothelium-dependent, acetylcholine-elicited, arterial smooth muscle relaxation (53, 58). The possible mechanisms by which acetylcholine elicits vascular smooth muscle relaxation are illustrated in Figure 2.

It may be of importance that endothelium-intact arteries relax to acetylcholine but endothelium-intact veins rarely undergo relaxation (57, 58). The reasons for this are unknown and may reflect the inability of venous endothelium to respond to acetylcholine with the generation of factors capable of causing relaxation. Alternatively, such factors may be generated but are less active in relaxing venous smooth muscle. The use of intrapulmonary arteries and the closely associated veins to compare the effects of acetylcholine and related agents shows promise in better understanding the differences in responsiveness of arteries and veins to endothelium-dependent vasoactive substances. The use of this technique in evaluating the effects of arachidonic acid and prostaglandins is described in the next section.

ARACHIDONIC ACID AND PROSTAGLANDINS

We have examined the effects of arachidonic acid and prostaglandins on intrapulmonary arteries and veins (59). The objective of these studies was to compare and contrast responses to acetylcholine and arachidonic acid because of suggestions that acetylcholine elicits relaxation by generating an endothelium-derived, lipoxygenase metabolite of arachidonic acid (53, 60, 61). Arachidonic acid causes relaxation of precontracted rings of endothelium-intact intrapulmonary artery by two distinct mechanisms (59). Extensive unpublished observations from this laboratory indicate that arachidonic acid stimulates the formation of both cyclic AMP and cyclic GMP. One component of relaxation and cyclic AMP production is inhibited by indomethacin, and methylene blue inhibits the second component of relaxation and cyclic GMP production. Endothelium-damaged arterial rings only contract in response to arachidonic acid or acetylcholine and show no changes in cyclic AMP levels. The contraction by arachidonic acid, but not by acetylcholine, is abolished by indomethacin. These data indicate that intrapulmonary arterial endothelium can generate at least two substances that elevate cyclic AMP and cyclic GMP levels. The endothelium-derived substance that causes cyclic AMP formation and relaxation in response to arachidonic acid appears to be prostacyclin, because PGE_2 elicits negligible effects and $PGF_{2\alpha}$ contracts, whereas prostacyclin markedly relaxes and elevates cyclic AMP levels in intrapulmonary artery. Veins with intact endothelium contract to both PGE_2 and $PGF_{2\alpha}$ and show little or no response to prostacyclin. The findings that arachidonic acid–elicited contractions of veins are abolished by indomethacin indicate that cyclooxy-

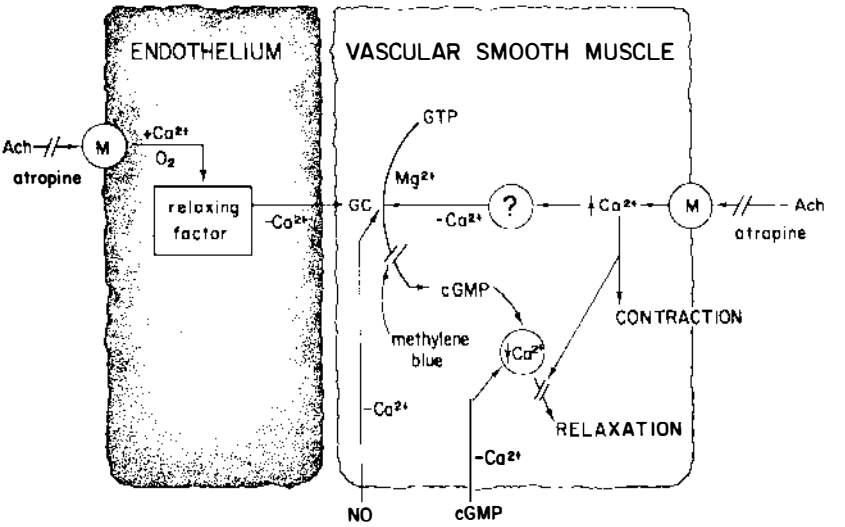

Figure 2 Schematic illustration of possible mechanism of vascular (arterial) smooth muscle relaxation produced by acetylcholine. Ach interacts with endothelial muscarinic receptors to generate a relaxing factor(s) in a Ca^{2+}- and O_2-dependent manner, which then permeates the smooth muscle cell to activate GC. The resulting elevated levels of cGMP promote intracellular Ca^{2+} sequestration, leading to muscle relaxation. Ach can also interact with smooth muscle muscarinic receptors to raise intracellular Ca^{2+} concentrations and cause contraction along with a small rise in cGMP levels. Abbreviations: Ach, acetylcholine; M, muscarinic receptor; NO, nitric oxide or nitrogen oxide–containing vasodilators; GC, guanylate cyclase; cGMP, cyclic GMP; $+Ca^{2+}$, calcium-dependent mechanism; $-Ca^{2+}$, calcium-independent mechanism; ?, unknown intermediate or mechanism. [Reproduced with permission from (57).]

genase products such as PGE_2 or $PGF_{2\alpha}$ may be responsible for this venous contraction. This view is supported by recent studies that microsomes isolated from intrapulmonary vein can synthesize significant quantities of PGE_2 (62). Moreover, both human (62) and bovine (D. B. McNamara, P. J. Kadowitz, unpublished information) intrapulmonary venous microsomes can generate significant amounts of PGI_2.

Since bovine intrapulmonary veins do not respond to prostacyclin in vitro, although they are clearly capable of forming it, it appears that arachidonic acid does not relax veins because these vessels are insensitive to prostacyclin. On the other hand, arteries markedly relax to both prostacyclin and its precursor arachidonic acid, and this is consistent with the observations that microsomes prepared from intrapulmonary artery can generate prostacyclin (62).

In view of the observations that acetylcholine relaxes endothelium-intact arterial rings in a cyclic AMP-independent manner, it appears inconceivable that acetylcholine elicits its marked relaxant response merely by releasing free arachidonic acid within endothelial cells. At concentrations that do not non-

selectively depress smooth muscle function, quinacrine does not inhibit cyclic GMP accumulation elicited by acetylcholine (57). Moreover, whereas arterial contractions by arachidonic acid are abolished by indomethacin, contractions to acetylcholine are not at all affected (53, 58). Endothelium-dependent arterial relaxation by acetylcholine may occur through activation of the lipoxygenase pathway or by mechanisms unrelated to arachidonic acid metabolism. The isolation and unequivocal identification of the proposed acetylocholine-stimulated, endothelium-derived relaxing factor(s) is essential prior to drawing any further conclusions.

PROBLEMS WITH NONSELECTIVE ANTAGONISTS

In general, experimentation on vascular smooth muscle employing relatively selective antagonists such as atropine, certain β-receptor blockers, methylene blue, and even indomethacin has yielded data that can be intrepreted unambiguously and with confidence. Other types of antagonists have been employed, however, and these data must be interpreted with caution. For example, quinacrine (also known as mepacrine) is an inhibitor of phospholipase A_2 activity and thereby reduces the liberation of arachidonic acid from phospholipid stores. Unfortunately, quinacrine possesses many other actions as well, including inhibition of certain effects of cyclic GMP (63) and nonselective depression of smooth muscle contractions through its local anesthetic action (41). In a recent study, the use of quinacrine to elucidate the mechanism of endothelium-dependent, acetylcholine-elicited, arterial relaxation proved fruitless (57). The same frustrations were experienced with several lipoxygenase and epoxygenase inhibitors (59). For example, in bovine intrapulmonary artery, nordihydroguiaretic acid elicits indomethacin-like effects on responses to arachidonic acid. The cytochrome P-450 inhibitor SKF 525-A also behaves like indomethacin, whereas another cytochrome P-450 inhibitor, metyrapone, is without effect.

A detailed and careful analysis of the effects of such enzyme inhibitors on vascular smooth muscle functions is mandatory before these chemicals can be used to assess the mechanisms of action of relaxing agents. Premature and incorrect conclusions will almost certainly result if such precautions are not followed. Nonselective inhibitors may not prove useful in assessing the nature of endothelium-derived relaxing substances.

THE PHYSIOLOGICAL IMPORTANCE OF CYCLIC GMP

Some of the most difficult questions to answer in the biological sciences pertain to the physiological relevance of proposed second messengers. Only after many years of research have the biological roles of cyclic AMP and calcium, for

example, become clearer. The possible physiological role of cyclic GMP constitutes a very recent development from only a limited number of laboratories. Recent evidence suggests that cyclic GMP is involved in the regulation of platelet aggregation (64, 65) and vascular smooth muscle relaxation (17–30, 41, 50, 51, 54–59). This chapter addresses the latter supposition.

Until recently, almost all the published work related to a possible second messenger role of cyclic GMP in expressing vascular smooth muscle relaxation elicited by exogenous chemicals, the nitrogen oxide–containing vasodilators. More recent work, however, hints that vasodilatation by endogenous neurotransmitters and autacoids may also be mediated by cyclic GMP. If this is true, then at least one physiological role for cyclic GMP becomes clear. Although endothelium-dependent relaxation by acetylcholine appears to involve cyclic GMP, several problems with the significance of these observations still exist. First, what is the source of acetylcholine in contact with endothelium in arteries that are at best only poorly innervated with cholinergic fibers, usually at the adventiomedial junction, a considerable distance from the endothelial cells? Second, what metabolic events occur in endothelial cells to render cyclic GMP accumulation in and relaxation of smooth muscle cells? Third, what is the mechanism by which intracellular cyclic GMP elicits vascular smooth muscle relaxation?

The importance of endothelial cells in arterial vasodilatation after parasympathetic nerve stimulation is unknown. Indeed, the contribution of neuronally released acetylcholine to vasodilatation has been debated in the past. Although responses to cholinergic stimulation appear to be modest in systemic vascular beds such as skeletal muscle (66), responses in the pulmonary vascular bed can be substantial under certain circumstances (67). Moreover, when pulmonary vascular tone is actively increased and the influence of adrenergic nerves in the vagosympathetic trunk are blocked, efferent vagal stimulation elicits marked frequency-dependent vasodilation (67), which is blocked by atropine but not by 5,8,11,14-eicosatetraynoic acid (67). Therefore, the physiological relevance of the in vitro observations that acetylcholine-elicited relaxation is endothelium-dependent is still uncertain. The only published study that comes close to addressing this problem is the recent observation that electrical field stimulation of norepinephrine-precontracted segments of intrapulmonary artery results in an endothelium-dependent relaxation (68). However, this relaxation was not affected by atropine, quinacrine, indomethacin, 5,8,11,14-eicosatetraynoic acid, tetrodotoxin, or procaine. Moreover, not only arteries but also veins relaxed in response to stimulation. Thus, a diffusible endothelium-derived relaxing factor is released by mechanisms unrelated to classical neurotransmitter release or arachidonic acid metabolism. Since acetylcholine is not involved here, the original question of the physiological importance of acetylcholine-elicited vasodilatation is even more important.

The nature of the endothelium-derived relaxing factor(s) generated by acetylcholine, electrical field stimulation, arachidonic acid, and various autocoids is unknown. Where studied, however, it is clear that cyclic GMP is involved in the relaxation process. It would be of great interest to know whether or not electrical field stimulation–elicited vascular smooth muscle relaxation is associated with concomitant increases in tissue cyclic GMP accumulation. In addition, it is clearly essential that the endothelium-derived factor(s) be isolated, purified, and characterized with respect to their capacity to stimulate cyclic GMP formation and relax vascular smooth muscle.

The mechanism by which cyclic GMP relaxes vascular smooth muscle needs to be understood prior to attempting to link cyclic GMP and relaxation in a cause-and-effect manner. At least one laboratory is currently attacking this problem by studying the properties of cyclic GMP–dependent protein kinase (69). Cyclic GMP itself relaxes vascular smooth muscle (70, 71), and sodium nitroprusside, a guanylate cyclase activator, leads to activation of cyclic GMP–dependent protein kinase in these tissues (72, 73). Cyclic GMP appears to antagonize the accumulation of free cytosolic calcium by intracellular mechanisms rather than by blocking extracellular influx of calcium (73). The first suggestion that cyclic GMP may regulate cellular calcium concentrations came as early as 1973 (2). One plausible theory is that cyclic GMP decreases intracellular free calcium concentrations in order to preserve normal cellular function (69). Other hypotheses are not ruled out. The mechanism by which cyclic GMP may elicit these effects could involve cyclic GMP–dependent protein kinase because large amounts of the kinase, as well as substrates for the kinase, exist in particulate material from vascular smooth muscle (74).

Certain autacoids and related substances now shown to require a functional endothelium in order to relax vascular smooth muscle have also been shown to stimulate the formation of cyclic GMP in these vessels. In addition to acetylcholine, researchers found that histamine and the calcium ionophore A23187 elevated cyclic GMP levels in rat thoracic aorta and that this was closely associated with relaxation (55). In other preliminary studies, A23187 and ultraviolet (UV) light were shown to elevate rabbit aortic levels of cyclic GMP and to elicit relaxation, both of which were dependent on endothelial cells (75, 76). It will be important to learn whether other autacoids that elicit endothelium-dependent relaxation also stimulate cyclic GMP formation in a temporally related manner.

Excellent correlations between vascular smooth muscle relaxation and cyclic GMP accumulation have been reported by several laboratories studying both nitrogen oxide–containing vasodilators and acetylcholine (see above). A recent report, however, suggested a poor correlation between relaxation and cyclic GMP accumulation when responses to nitroglycerin and acetylcholine were compared (56). That is, nitroglycerin caused more relaxation for a given

increment in cyclic GMP levels than did acetylcholine. Based on these and other observations, the authors of the report concluded that nitroglycerin may relax rabbit aorta by more than one mechanism. Perhaps a better and more obvious explanation for these apparently discrepant findings is simply that the concentration of intracellular calcium is a critical determinant of the degree of relaxation but not of cyclic GMP accumulation. It is fairly widely appreciated that nitroglycerin elicits relaxation and cyclic GMP accumulation in a calcium-independent manner, whereas acetylcholine has an absolute requirement for calcium. Acetylcholine raises intracellular calcium concentrations whereas nitroglycerin does not, and elevated calcium ion may tend to attenuate the relaxations to acetylcholine or any other relaxant. It is important to appreciate that acetylcholine still partially elevates cyclic GMP levels while contracting endothelium-damaged arteries (57). Thus, nitroglycerin and related agents would be expected to elicit greater relaxation at a given degree of cyclic GMP accumulation than would acetylcholine. This interpretation is supported by observations that lowering calcium concentrations in vascular smooth muscle results in the enhancement of relaxation by sodium nitroprusside and 8-bromo-cyclic GMP (73).

Over the years, good experimental evidence has accumulated to suggest that cyclic AMP is a second messenger in expressing the relxant effects of agents that activate hormone-sensitive adenylate cyclase. These agents include epinephrine, isoproterenol, other catecholamines, prostacyclin, other prostaglandins, and related neurohormonal substances and autacoids (27, 28). One unresolved question is why do both cyclic AMP and cyclic GMP seem to be involved in mediating or modulating vascular smooth muscle relaxation? Figure 3 attempts to illustrate schematically the possible complementary physiological roles of both cyclic nucleotides in vasodilatation.

CONCLUSIONS, HYPOTHESIS, AND EVIDENCE

Based on the available experimental evidence, discussed above, we offer the working hypothesis that cyclic GMP plays an essential physiological role in modulating vascular smooth muscle tone. The evidence that cyclic GMP is intimately involved in vascular smooth muscle relaxation elicited by a variety of vasodilators can be summarized as follows.

1. Nitrogen oxide–containing vasodilators, as well as acetycholine and certain related agents, produce concentration- and time-dependent increases in vascular cyclic GMP levels that are associated temporally with relaxation;
2. Activators of soluble guanylate cyclase cause both cyclic GMP accumulation in and relaxation of arteries and veins;
3. Inhibitors of guanylate cyclase (which permeate cells, i.e. methylene blue)

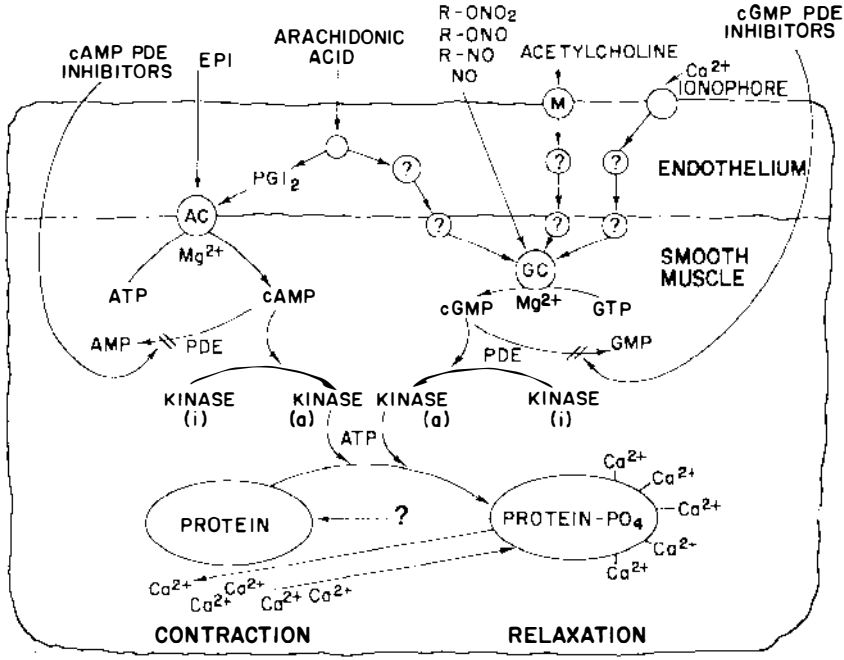

Figure 3 Schematic illustration of the possible physiological role of cyclic AMP and cyclic GMP in vascular smooth muscle relaxation. cAMP levels are increased by activation of AC in smooth muscle or by inhibition of PDE. Similarly, cGMP levels are increased by activation of GC (by endothelium-dependent or independent mechanisms) or by inhibition of PDE. Arachidonic acid can stimulate formation of both cAMP and cGMP by endothelium-dependent mechanisms. cAMP and cGMP activate their own specific, cyclic nucleotide–dependent protein kinases, resulting in the phosphorylation of one or more similar proteins. The phosphorylated form of the protein(s) rapidly binds intracellular Ca^{2+}, thereby leading to smooth muscle relaxation. Dephosphorylation of protein-PO_4 may release bound Ca^{2+} and enhance tone (or attenuate relaxation). Abbrevations: EPI, epinephrine; R-ONO_2, organic nitrates; R-ONO, organic nitrites; R-NO, organic nitroso compounds; NO, nitric oxide, cAMP, cyclic AMP; cGMP, cyclic GMP; AC, hormone-sensitive adenylate cyclase; GC, soluble guanylate cyclase; ?, unknown intermediate or mechanism.

cause marked inhibition or abolition of both cyclic GMP accumulation and relaxation elicited by nitrogen oxides or acetycholine;

4. Cyclic GMP phosphodiesterase inhibitors potentiate both cyclic GMP accumulation and relaxation in response to the above vasodilators;
5. Cyclic GMP itself or more lipophilic analogs directly relax vascular smooth muscle.

Additional evidence is amassing that cyclic GMP may be important for regulating vascular smooth muscle tone. This is summarized as follows.

1. The guanylate cyclase inhibitor methylene blue (also brilliant cresyl blue), when used in concentrations exceeding 0.1 mM, lowers resting levels of

cyclic GMP and causes vascular smooth muscle contraction, both of which are readily reversed by inhibitors of cyclic GMP phosphodiesterase;
2. Guanylate cyclase inhibitors markedly enhance contractions in response to α-receptor agonists, KCl and acetylcholine in veins or in endothelium-damaged arteries, whereas cyclic GMP phosphodiesterase inhibitors attenuate such contractions;
3. Guanylate cyclase activators stimulate cyclic GMP–dependent protein kinase in smooth muscle, and these tissues contain substrates for the kinase;
4. Relaxants that elevate cyclic GMP levels also stimulate phosphorylation of endogenous proteins;
5. Lowering calcium concentrations in vascular smooth muscle enhances relaxation responses to added cyclic GMP or guanlyate cyclase activators.

The successful design and execution of the following experiments could add considerable support to the hypothesis that cyclic GMP modulates, and may be obligatory for, vasodilatation by nitrogen oxide–containing drugs, acetylcholine, and closely related vasodilators.

1. The isolation, identification, and characterization of endothelium-derived relaxing factors, which may be different for different hormone-like, calcium-dependent vasodilators;
2. The isolation and characterization of calcium-binding proteins from vascular smooth muscle, which may be the principal substrates for cyclic GMP–dependent protein kinase in these tissues;
3. The development of specific antibodies to soluble guanylate cyclase and/or cyclic GMP-dependent protein kinase in order to ascertain the absolute involvement of these metabolic pathways in vasodilatation under a variety of test conditions.

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Literature Cited

1. Lee, T. P., Kuo, J. F., Greengard, P. 1972. Role of muscarinic cholinergic receptors in regulation of guanosine 3',5'-cyclic monophosphate content in mammalian brain, heart muscle, and intestinal smooth muscle. *Proc. Natl. Acad. Sci. USA* 69:3287–91
2. Schultz, G., Hardman, J. G., Sutherland, E. W. 1973. Cyclic nucleotides and smooth muscle function. In *Asthma, Physiology, Immunopharmacology, and Treatment*, ed. K. F. Austen, L. M. Lichtenstein, pp. 123–38. New York: Academic
3. Dunham, E. W., Haddox, J. K., Goldberg, N. D. 1974. Alteration of vein cyclic 3',5'-nucleotide concentrations during changes in contractility. *Proc. Natl. Acad. Sci. USA* 71:815–19
4. Andersson, R., Nilsson, K., Wikberg, J., Johansson, S., Mohme-Lundholm, E., Lundholm, L. 1975. Cyclic nucleotides and the contraction of smooth muscle. *Adv. Cyclic Nucl. Res.* 5:491–518

5. Clyman, R. I., Sandler, J. A., Manganiello, V. C., Vaughan, M. 1975. Guanosine 3',5'-monophosphate and adenosine 3',5'-monophosphate content of human umbilical artery. *J. Clin. Invest.* 55:1020–25
6. Spies, C., Schultz, K. D., Schultz, G. 1980. Inhibitory effects of mepacrine and eicosatetraynoic acid on cyclic GMP elevations caused by calcium and hormonal factors in rat ductus deferens. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 311:71–77
7. Diamond, J., Holmes, T. G. 1975. Effects of potassium chloride and smooth muscle relaxants on tension and cyclic nucleotide levels in rat myometrium. *Can. J. Physiol. Pharmacol.* 53:1099–107
8. Diamond, J., Blisard, K. S. 1976. Effects of stimulant and relaxant drugs on tension and cyclic nucleotide levels in canine femoral artery. *Mol. Pharmacol.* 12:688–92
9. Kimura, H., Mittal, C. K., Murad, F. 1975. Increases in cyclic GMP levels in brain and liver with sodium azide an activator of guanylate cyclase. *Nature* 257:700–2
10. Kimura, H., Mittal, C. K., Murad, F. 1975. Activation of guanylate cyclase from rat liver and other tissues by sodium azide. *J. Biol. Chem.* 250:8016–22
11. Katsuki, S., Arnold, W., Mittal, C., Murad, F. 1977. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucl. Res.* 3:23–35
12. Schultz, K. D., Schultz, K., Schultz, G. 1977. Sodium nitroprusside and other smooth muscle relaxants increase cyclic GMP levels in rat ductus deferens. *Nature* 265:750–51
13. Katsuki, S., Murad, F. 1977. Regulation of adenosine cyclic 3',5'-monophosphate and guanosine cyclic 3'5'-monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.* 13:330–41
14. Bohme, E., Graf, H., Schultz, G. 1978. Effects of sodium nitroprusside and other smooth muscle relaxants on cyclic GMP formation in smooth muscle and platelets. *Adv. Cyclic Nucl. Res.* 9:131–43
15. Diamond, J. 1978. Role of cyclic nucleotides in control of smooth muscle contraction. *Adv. Cyclic Nucl. Res.* 9:327–40
16. Diamond, J. 1983. Lack of correlation between cyclic GMP elevation and relaxation of nonvascular smooth muscle by nitroglycerin, nitroprusside, hydroxylamine and sodium azide. *J. Pharmacol. Exp. Ther.* 225:422–26
17. Gruetter, C. A., Barry, B. K., McNamara, D. B., Gruetter, D. Y., Kadowitz, P. J., Ignarro, L. J. 1979. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. *J. Cyclic Nucl. Res.* 5:211–24
18. Gruetter, C. A., Barry, B. K., McNamara, D. B., Kadowitz, P. J., Ignarro, L. J. 1980. Coronary arterial relaxation and guanylate cyclase activation by cigarette smoke, N'-nitrosonornicotine and nitric oxide. *J. Pharmacol. Exp. Ther.* 214:9–15
19. Gruetter, C. A., Kadowitz, P. J., Ignarro, L. J. 1981. Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrite and amyl nitrite. *Can. J. Physiol. Pharmacol.* 59:150–56
20. Gruetter, C. A., Gruetter, D. Y., Lyon, J. E., Kadowitz, P. J., Ignarro, L. J. 1981. Relationship between cyclic GMP formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. *J. Pharmacol. Exp. Ther.* 219:181–86
21. Ignarro, L. J., Lippton, H. L., Edwards, J. C., Baricos, W. H., Hyman, A. L., et al. 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* 218:739–49
22. Lippton, H. L., Gruetter, C. A., Ignarro, L. J., Meyer, R. L., Kadowitz, P. J. 1982. Vasodilator actions of several N-nitroso compounds. *Can. J. Physiol. Pharmacol.* 60:68–75
23. Ignarro, L. J., Gruetter, C. A. 1980. Requirement of thiols for activation of coronary arterial guanylate cyclase by glyceryl trinitrate and sodium nitrite: Possible involvement of S-nitrosothiols. *Biochim. Biophys. Acta* 631:221–31
24. Edwards, J. C., Ignarro, L. J., Hyman, A. L., Kadowitz, P. J. 1984. Relaxation of intrapulmonary artery and vein by nitrogen oxide–containing vasodilators and cyclic GMP. *J. Pharmacol. Exp. Ther.* 228:33–42
25. Axelsson, K. L., Wikberg, J. E. S., Andersson, R. G. G. 1979. Relationship

between nitroglycerin, cyclic GMP and relaxation of vascular smooth muscle. *Life Sci.* 24:1779–86

26. Kukovetz, W. R., Holzmann, S., Wurm, A., Poch, G. 1979. Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 310:129–38
27. Kukovetz, W. R., Poch, G., Holzman, S., Wurm, A., Rinner, I. 1979. Cyclic nucleotides and coronary flow. In *Cyclic Nucleotides and Therapeutic Perspectives,* ed. G. Cehovic, G. A. Robison, pp. 109–25. Oxford: Pergamon
28. Kukovetz, W. R., Poch, G., Holzmann, S. 1981. Cyclic nucleotides and relaxation of vascular smooth muscle. In *Vasodilatation,* ed. P. M. Vanhoutte, I. Leusen, pp. 339–53. New York: Raven
29. Keith, R. A., Burkman, A. M., Sokoloski, T. D., Fertel, R. H. 1982. Vascular tolerance to nitroglycerin and cyclic GMP generation in rat aortic smooth muscle. *J. Pharmacol. Exp. Ther.* 221:525–31
30. Galvas, P. E., DiSalvo, J. 1983. Concentration and time-dependent relationships between isosorbide dinitrate-induced relaxation and formation of cyclic GMP in coronary arterial smooth muscle. *J. Pharmacol. Exp. Ther.* 224:373–78
31. Arnold, W. P., Mittal, C. K., Katsuki, S., Murad, F. 1977. Nitric oxide activates guanylate cyclase and increases guanosine 3',5'-cyclic monophosphate levels in various tissue preparations. *Proc. Natl. Acad. Sci. USA* 74:3203–7
32. DeRubertis, F. R., Craven, P. A. 1976. Calcium-independent modulation of cyclic GMP and activation of guanylate cyclase by nitrosoamines. *Science* 193:897–99
33. Craven, P. A., DeRubertis, F. R. 1978. Restoration of the responsiveness of purified guanylate cyclase to nitrosoguanidine, nitric oxide, and related activators by heme and heme proteins: Evidence for the involvement of the paramagnetic nitrosyl-heme complex in enzyme activation. *J. Biol. Chem.* 253:8433–43
34. Schoental, R., Rive, D. J. 1965. Interaction of N-alkyl-N-nitrosourethanes with thiols. *Biochem. J.* 97:466–74
35. McCalla, D. R., Reuvers, A., Kitai, R. 1968. Inactivation of biologically active N-methyl-N-nitroso compounds in aqueous solution: Effect of various conditions of pH and illumination. *Can. J. Biochem.* 46:807–11
36. Schulz, U., McCalla, D. R. 1969. Reactions of cysteine with N-methyl-N-nitroso-p-toluenesulfonamide and N-methyl-N'-nitro-N-nitrosoguanidine. *Can. J. Chem.* 47:2021–27
37. Lawley, P. D., Thatcher, C. J. 1970. Methylation of deoxyribonucleic acid in cultured mammalian cells by N-methyl-N'-nitro-N-nitrosoguanidine. *Biochem. J.* 116:693–707
38. Ignarro, L. J., Edwards, J. C., Gruetter, D. Y., Barry, B. K., Gruetter, C. A. 1980. Possible involvement of S-nitrosothiols in the activation of guanylate cyclase by nitroso compounds. *FEBS Letts.* 110:275–78
39. Ignarro, L. J., Barry, B. K., Gruetter, D. Y., Edwards, J. C., Ohlstein, E. H., et al. 1980. Guanylate cyclase activation by nitroprusside and nitrosoguanidine is related to formation of S-nitrosothiol intermediates. *Biochem. Biophys. Res. Commun.* 94:93–100
40. Murad, F., Mittal, C. K., Arnold, W. P., Katsuki, S., Kimura, H. 1978. Guanylate cyclase activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. *Adv. Cyclic Nucl. Res.* 9: 145–58
41. Ignarro, L. J., Gruetter, C. A., Hyman, A. L., Kadowitz, P. J. 1983. Molecular mechanisms of vasodilatation. In *Dopamine Receptor Agonists,* ed. G. Poste, S. T. Crooke, pp. 259–88. New York: Plenum
42. Craven, P. A., DeRubertis, F. R., Pratt, D. W. 1979. Electron spin resonance study of the role of NO-catalase in the activation of guanylate cyclase by NaN_3 and NH_2OH: Modulation of enzyme responses by heme protein and their nitrosyl derivatives. *J. Biol. Chem.* 254:8213–22
43. Ignarro, L. J., Degnan, J. N., Baricos, W. H., Kadowitz, P. J., Wolin, M. S. 1982. Activation of purified guanylate cyclase by nitric oxide requires heme: Comparison of heme-deficient, heme-reconstituted and heme-containing forms of soluble enzyme from bovine lung. *Biochim. Biophys. Acta* 718:45–59
44. Wolin, M. S., Wood, K. S., Ignarro, L. J. 1982. Guanylate cyclase from bovine lung: A kinetic analysis of the regulation of the purified soluble enzyme by protoporphyrin IX, heme and nitrosyl-heme. *J. Biol. Chem.* 257:13312–20
45. Ignarro, L. J., Wood, K. S., Wolin, M. S. 1984. Regulation of purified soluble guanylate cyclase by porphyrins and metalloporphyrins: A unifying concept. *Adv. Cyclic Nucl. Res.* 17:267–74
46. Kukovetz, W. R., Holzmann, S., Poch,

G. 1982. Function of cyclic GMP in acetylcholine-induced contraction of coronary smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 319: 29–33
47. Kadowitz, P. J., Nandiwada, P., Gruetter, C. A., Ignarro, L. J., Hyman, A. L. 1981. Pulmonary vasodilator responses to nitroprusside and nitroglycerin in the dog. *J. Clin. Invest.* 67:893–902
48. Needleman, P., Johnson, E. M. 1973. Mechanism of tolerance development to organic nitrates. *J. Pharmacol. Exp. Ther.* 184:709–15
49. Needleman, P., Jakschik, B., Johnson, E. M. 1973. Sulfhydryl requirement for relaxation of vascular smooth muscle. *J. Pharmacol. Exp. Ther.* 187:324–31
50. Axelsson, K. L., Andersson, R. G. G., Wikberg, J. E. S. 1982. Vascular smooth muscle relaxation by nitro compounds: Reduced relaxation and cyclic GMP elevation in tolerant vessels and reversal of tolerance by dithiothreitol. *Acta Pharmacol. Toxicol.* 50:350–57
51. Axelsson, K. L., Andersson, R. G. G. 1983. Tolerance towards nitrogylcerin, induced in vivo, is correlated to a reduced cGMP response and an alteration in cGMP turnover. *Eur. J. Pharmacol.* 88:71–79
52. Horowitz, J. D., Antman, E. M., Lorell, B. H., Barry, W. H., Smith, T. W. 1982. Potentiation of cardiovascular effects of nitroglycerin by N-acetylcysteine. *Circulation* 66(2):II–264
53. Furchgott, R. F., Zawadzki, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–76
54. Holzmann, S. 1982. Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. *J. Cyclic Nucl. Res.* 8:409–19
55. Rapoport, R. M., Murad, F. 1983. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.* 52:352–57
56. Diamond, J., Chu, E. B. 1983. Possible role for cyclic GMP in endothelium-dependent relaxation in rabbit aorta by acetylcholine. Comparison with nitroglycerin. *Res. Commun. Chem. Pathol. Pharmacol.* 41(3):369–81
57. Ignarro, L. J., Burke, T. M., Wood, K. S., Wolin, M. S., Kadowitz, P. J. 1984. Association between cyclic GMP accumulation and acetylcholine-elicited relaxation of bovine intrapulmonary artery. *J. Pharmacol. Exp. Ther.* 228:682–90
58. Furchgott, R. F. 1984. The role of endothelium in the responses of vascular smooth muscle to drugs. *Ann. Rev. Pharmacol. Toxicol.* 24:175–97
59. Ignarro, L. J., Harbison, R., Burke, T., Wolin, M., Kadowitz, P. J. 1984. Endothelium-dependent relaxation of bovine intrapulmonary artery by arachidonic acid. Involvement of two distinct mechanisms. *Fed. Proc.* 43:737
60. Furchgott, R. F., Zawadzki, J. V., Cherry, P. D. 1981. Role of endothelium in the vasodilator response to acetylcholine. See Ref. 28, pp. 49–66
61. Furchgott, R. F. 1983. Role of endothelium in responses of vascular smooth muscle. *Circ. Res.* 53:557–73
62. McMullen-Laird, M., McNamara, D. B., Kerstein, M. D., Hyman, A. L., Kadowitz, P. J. 1982. Human lung metabolism of prostaglandin endoperoxide. *Circulation* 66(2):II–166
63. Greenberg, R. N., Guerrant, R. L., Chang, B., Robertson, D. C., Murad, F. 1982. Inhibition of *Escherichia coli* heat-stable enterotoxin effects on intestinal guanylate cyclase and fluid secretion by quinacrine. *Biochem. Pharmacol.* 31: 2005–9
64. Mellion, B. T., Ignarro, L. J., Ohlstein, E. H., Pontecorvo, E. G., Hyman, A. L., Kadowitz, P. J. 1981. Evidence for the inhibitory role of cylic GMP in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. *Blood* 57:946–55
65. Mellion, B. T., Ignarro, L. J., Meyers, C. B., Ohlstein, E. H., Ballot, B. A., et al. 1983. Inhibition of human platelet aggregation by S-nitrosothiols: Heme-dependent activation by soluble guanylate cyclase and stimulation of cyclic GMP accumulation. *Mol. Pharmacol.* 23:653–64
66. Brody, M. J., Shaffer, R. A. 1970. Distribution of vasodilator nerves in the canine hindlimb. *Am. J. Physiol.* 218: 470–74
67. Nandiwada. P. A., Hyman, A. L., Kadowitz, P. J. 1983. Pulmonary vasodilator responses to vagal stimulation and acetylcholine in the cat. *Circ. Res.* 53:86–95
68. Frank, G. W., Bevan, J. A. 1983. Electrical stimulation causes endothelium-dependent relaxation in lung vessels. *Am. J. Physiol.* 244:H793–98
69. Lincoln, T. M., Corbin, J. D. 1983. Characterization and biological role of the cGMP-dependent protein kinase. *Adv. Cyclic Nucl. Res.* 15:139–92
70. Schultz, K. D., Bohme, E., Kreye, V. A.

W., Schultz, G. 1979. Relaxation of hormonally stimulated smooth muscular tissues by the 8-bromo derivative of cyclic GMP. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 306:1–9

71. Napoli, S. A., Gruetter, C. A., Ignarro, L. J., Kadowitz, P. J. 1980. Relaxation of bovine coronary arterial smooth muscle by cyclic GMP, cyclic AMP and analogs. *J. Pharmacol. Exp. Ther.* 212:469–73
72. Rapoport, R. M., Draznin, M. B., Murad, F. 1982. Sodium nitroprusside-induced protein phosphorylation in intact rat aorta is mimicked by 8-bromo cyclic GMP. *Proc. Natl. Acad. Sci. USA* 79:6470–74
73. Lincoln. T. M. 1983. Effects of nitroprusside and 8-bromo-cyclic GMP on the contractile activity of the rat aorta. *J. Pharmacol. Exp. Ther.* 224:100–7
74. Casnellie, J. E., Schlichter, D. J., Walter, U., Greengard, P. 1978. Photoaffinity labeling of a guanosine 3':5'-monophosphate-dependent protein kinase from vascular smooth muscle. *J. Biol. Chem.* 253:4771–76
75. Martin, W., Villani, G. M., Furchgott, R. F. 1984. Hemoglobin and methylene blue selectively inhibit relaxation of rabbit aorta by agents which increase cyclic GMP levels. *Fed. Proc.* 43:737
76. Furchgott, R. F., Jothianandan, D. 1984. Relaxation of rabbit aorta by light is associated with an increase in cyclic GMP. *Fed. Proc.* 43:737