The Interaction between vasopressin and modulators of the cardiovascular system

Streefkerk, J.O.

Citation for published version (APA):
CHAPTER 5

INFLUENCE OF THE NATURE OF PRECONTRACTION ON THE RESPONSES TO COMMONLY EMPLOYED VASODILATOR AGENTS IN RAT ISOLATED AORTIC RINGS.

Fundamental and Clinical Pharmacology 2002;16: 485-494
1. Introduction

Vasodilator activity of various drugs continues to be of considerable therapeutic interest. Its qualitative and quantitative evaluation is the main goal of numerous ongoing in vivo and in vitro studies. Although the objective of these studies seems to be obvious, their execution is far from that. The mechanisms by which vessels can be dilated are manifold. They range from the inhibition of voltage- or receptor-operated calcium channels to the generation or accumulation of intracellular second messengers, that initiate a series of events resulting in the sequestration of activator calcium and/or a desensitisation of the contractile elements.

Acute vasodilation in vitro can only be induced and measured after activation of vascular smooth muscle preparations. Measured vasodilator activity, however, may depend on the type and the magnitude of smooth muscle activation. In principle, there exist two major mechanisms leading to an increase of muscular activity: 1) the influx of calcium ions following their huge concentration gradient over the plasma membrane through potential- or receptor-operated calcium-specific channels and 2) the activation of the intracellular membrane bound enzyme phospholipase C, which cleaves phosphatidylinositol-4,5-bisphosphate resulting into the formation of inositol-1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ induces the release of calcium ions from intracellular stores, thus activating the contractile elements. It seems reasonable to assume that the increase in intracellular free calcium concentration as well as its elimination might be different between the aforementioned two mechanisms. Such differences may influence the potency and the efficacy of counteracting vasodilators.

Another problem is the fact that the signalling indicator system, that is the contraction of the smooth muscle cells, is only partially reflecting the underlying biochemical events. Any contractile response is by definition limited to the maximal possible activation of the contractile elements on the one hand and the complete loss of all muscular activity on the other hand. An increase of the intracellular free calcium concentration beyond the binding capacity of troponin will not result in any further contraction, although the maximal capacity e.g. of an agonist to liberate calcium ions might not be reached. On the other hand the vasodilator activity of any pharmacological measure
becomes only visible if there exists an active muscular tension. If any smooth muscle contraction is abolished a further increase of the concentration of vasodilator second messengers like cyclic GMP or cyclic AMP, will not result in any observable effect. These limitations of the signalling indicator system, although of utmost physiological relevance, will obscure the true concentration-response relationships of vasodilators as well as of vasoconstrictors. This issue has been critically adressed by Lew et al. 7.

An important practical problem arises from these considerations: To which degree does the type and the magnitude of the precontraction influence the concentration-response relationship of a given vasodilator?

In order to secure the comparability and thereby reliability of in vitro studies that investigate vasodilator drugs, evaluation of the methodology used in these studies is necessary. In the present investigation we therefore aim to determine to what extent the vasorelaxing properties of vasodilators drugs in-vitro depend on the qualitative and quantitative characteristics of the applied pre-contraction. In order to do so we performed in-vitro experiments in rat aortic rings and constructed concentration-response curves to various vasodilators after pre-contraction with different concentrations of commonly employed vasoconstrictor agents.

2. Methods

Aortic ring preparations

Wistar rats (Charles River) weighing 240-260g were sacrificed by stunning and exsanguination. The thoracic aorta was carefully excised and placed in a physiological salt solution (PSS) of the following composition (mM): NaCl (118.5); KCl (4.7); KH₂PO₄ (1.2); CaCl₂ (2.5); MgSO₄ (1.2); glucose (5.5); Na₄EDTA (0.026); NaHCO₃ (25) at room temperature, which was continuously gassed with carbogen (95% O₂ and 5% CO₂). The aortas were cleaned of superficial fat and connective tissue. Care was taken not to stretch the vessels or to damage the endothelium. Out of one aorta three segments were prepared in which the three precontraction doses in succession were evaluated for each vasodilator agent. The aortic rings were cut into segments of approximately 3 mm long each, and mounted between two triangular stainless steel hooks and put into organ baths containing 5 ml PSS at 37°C. The PSS
was continuously gassed with carbogen. Isometric tension was measured by means of isometric force transducers (A.D. instruments, Castle Hill, Australia), connected to a MacLab/8 computer system. The aortic rings were equilibrated in PSS for 30 min at a resting tension of 1.0 g, which was maintained throughout the experiment.

**Experimental protocol**

After the equilibration period the aortic rings were exposed three times for 5 min. to a depolarising PSS (containing 60 mM K+) thus causing a contraction of the vascular smooth muscle cells. In this PSS solution 55.3 mM NaCl has been isotonicall y replaced by KCl. In between the K+-induced there were equilibration intervals of 20 min. Between the second and third contraction, the vessels were subjected to vasoconstriction, induced by PhE 0.1 μM, after which endothelial function was tested by means of MCh (0.3, 1 and 3 μM respectively). Preparations with an endothelial function (E_{max}) of less than 80 % were considered too much damaged for the experiment and were discarded.

Thirty minutes after the last potassium-induced contraction, the aortic rings were pre-contracted to different levels with various vasoconstrictors in 3 or 4 different concentrations. Thereafter concentration-response curves were constructed for the vasodilators under investigation. The concentration response-curves of these vasodilators were compared by means of calculated pD2 and E_{max} values and correlated by the characteristics of the preceding precontraction. We used the following three vasoconstrictor agents in three concentrations for the precontraction: phenylephrine (PhE) 0.1, 0.3 and 3 μM, U46619 (U-46) 0.18, 0.3 and 1 μM and increasing concentrations of potassium ions (25, 30 and 40 mM), respectively. Again, in the latter group of experiments, sodium in the PSS had been replaced by an isotonically equivalent concentration of potassium. The lowest concentration of each compound applied is the lowest concentration needed for a stable contraction throughout the experiment; while the highest concentration applied is the concentration needed to reach maximal effect (E_{max} of 100%). After induction of these different precontractions, concentration-response curves were constructed for the following vasodilators: sodium nitroprusside (SNP), methacholine (MCh) and forskolin (FSK), respectively. The rationale behind the chosen agents is elucidated in the first part of the discussion.
As many contractile agents are known to induce the release of NO, a separate set of experiments was performed. We compared the different concentrations of PhE and K⁺-ions and their influence on the SNP concentration response curve, in the presence or absence of the NO-synthesis inhibitor L-NAME. In experiments with L-NAME, a concentration of 100 μM L-NAME was maintained throughout the experiment.

Furthermore to investigate the influence of extracellular Ca²⁺-influx on the various vasodilator effects produced by the successive levels of precontraction, we constructed a concentration response curve for the L-type Ca²⁺-channel blocker nifedipine, after a pre-contraction with several concentrations of the partial α₁-adrenoceptor agonist St 587. We used St 587 because the contractile response to this agonist is mainly dependent on extracellular Ca²⁺. In the rat aortic rings the endothelium had to be removed in order to produce a stable St 587 contraction.

![Figure 1](image)

**Figure 1.** Contractile responses in rat isolated aorta preparations to phenylephrine: 0.1, 0.3 and 3 μM; U46619: 0.18, 0.3 and 1 μM; St 587 1, 3, 10 and 30 μM, and potassium ions 25, 30 and 40 mM. Values are expressed as means ± SEM. * p < 0.05 compared to the lowest concentration of the contractile compound. $ p< 0.05 compared to the same concentration of the contractile compound without L-NAME.
**Drugs used**

L-phenylephrine hydrochloride, U-46619, acetyl-β methacholine chloride and nifedipine were obtained from Sigma chemical Co. (St. Louis, MO, USA) and sodium-nitroprusside from Merck (Darmstadt, Germany). Forskolin and St 587 were kindly donated by Hoechst (Amsterdam, the Netherlands) and Boehringer (Ingelheim, Germany) respectively. N-omega-nitro-L-Arginine methyl ester HCl was purchased from ICN (Aurora, Ohio, USA). Forskolin was dissolved in 100% DMSO to a concentration of 10 mM, and the solution was subsequently diluted with a mixture of propylene glycol: DMSO (9:1) to a concentration of 5 mM. For lower concentrations, this solution was diluted with distilled water. The final concentration of DMSO in the medium proved to have no effect on the measurements (data not shown). All other drugs were dissolved in distilled water to a stock solution of 10 mM; the stock-solutions were diluted with distilled water to obtain the final drug concentration.

**Statistical evaluation**

The data are expressed as means ± S.E.M. for at least 6 measurements. The concentration-response curves for the compounds investigated were analysed by means of a computer program (Graph Pad, Institute for Scientific Informatics, San Diego, CA, U.S.A.). The pD₂-value [-log effective concentration (molar) that produces 50% of the maximal inhibitory effect (IC₅₀)], as well as the maximal effect (Eₘₐₓ) were obtained from the non-linear regression curve fit analysis for the individual set of experiments. The statistical significance of the differences was evaluated using a one-way analysis of variance followed by a Tukey post test. Values of P<0.05 were considered significant.

**3. Results**

As shown in figure 1 vasoconstrictor responses to several pharmacological stimulants were concentration dependent. All agonists applied, produced stable contractions. Subsequently, vasodilator drugs were administered after pre-contraction with the different vasoconstrictor agents. For each level of pre-contraction induced by a particular vasoconstrictor a concentration response
The curve (CRC) of the vasodilator agent investigated was constructed. The CRC obtained for various vasodilator drugs are visualized in the figures 2-4.

**Figure 2.** Influence of the precontraction by phenylephrine (PhE) (0.1, 0.3 and 3 μM) on the concentration response curve of (a) sodium nitroprusside and (b) methacholine. Values are expressed as means ± SEM. * p < 0.05, ** p<0.01 compared to PhE 0.1 μM.
The cumulative CRC for sodium nitroprusside (SNP), showed a potency (pD$_2$ value) of 8.56 ± 0.13 after pre-contraction with PhE 0.1μM (Figure 2a). For the subsequent concentration PhE (0.3 μM) the potency showed a tendency to decrease. This decrease was evident after PhE 3 μM, the highest concentration evaluated, where the potency decreased 16 fold to a value of 7.35 ± 0.09 (p<0.01).

The relaxation response to methacholine (MCh) (Figure 2b) demonstrated similar results regarding the potency and efficacy at the two lowest concentrations of PhE. With respect to the highest 3μM concentration PhE, the potency for MCh remained comparable to that observed at the two lower concentrations, but a significantly (p < 0.01) decreased relaxation to 43.3 % ± 6.9 % of the basal pre-contractile force was found.

Responses to forskolin (FSK) appeared to be independent of the pre-contracting concentrations of PhE. Data are not shown.
Figure 3. Influence of the precontraction by U-46619 (U-46) (0.18, 0.3 and 1 μM) on the concentration response curve of (a) sodium nitroprusside (b) methacholine and (c) forskolin. Values are expressed as means ± SEM. * p < 0.05, ** p<0.01 compared to U-46 0.18 μM.

After pre-contractions induced by 0.18, 0.3 and 1μM U-46, respectively, the potency of SNP (Figure 3a) decreased from 7.61 ± 0.11, to 7.15 ± 0.22 (p<0.05), and finally to 6.57 ± 0.44 (p<0.05), and the efficacy decreased from 84 % ± 4.4 %, to 71 % ± 9.2 % to 17 % ± 8.8 % (p<0.05), respectively.
The rightward shift observed in the phenylephrine-nitroprusside curves was also found after pre-contraction with U-46. The major difference when compared to the phenylephrine-nitroprusside experiments is the decreased $E_{\text{max}}$ value achieved after U-46 pre-contraction.

The MCh CRC (Figure 3b), established after the subsequent U-46 concentrations of 0.18 μM, 0.3 μM and 1 μM, revealed comparable potencies for the lowest two concentrations, but the efficacy of 64.5 % ± 12.3 % (U-46 0.18 μM) was abolished to 0.0 % ± 9.2 % (p<0.05) after the highest concentration of U-46. When compared to the phenylephrine-methacholine experiments the submaximal pre-contractions towards U-46 could not be entirely reduced to baseline values and the response to the highest concentration U-46 (1 μM) could not be influenced at all.

The FSK (Figure 3c) CRC, following U-46 pre-contraction, proved comparable with the phenylephrine-forskolin CRC, with respect to the efficacies, which were also approximately 100 %. However, the potency proved to be decreased (p<0.05) from $7.91\pm0.26$ to $7.15\pm0.10$ for the lowest and highest concentrations investigated, respectively.

![Graph](image)
Vasodilator drugs and pre-contraction in vitro

Figure 4. Influence of the precontraction by potassium (K⁺) (25, 30 and 40 mM) on the concentration response curve of (a) sodium nitroprusside (b) methacholine and (c) forskolin. Values are expressed as means ± SEM. * p < 0.05, ** p<0.01 compared to K⁺ 25 mM.

In the SNP/K⁺ experiments (Figure 4a) potassium ions at 30 and 40 mM caused a decrease of potency compared to the lowest K⁺ concentration (25
mM) applied. The SNP potency decreased from 8.7±0.1 to 8.2±0.1 (p<0.05) and further to 7.2±0.1 (p<0.05) for the increasing potassium concentrations. The efficacy of SNP was only decreased after the highest concentration of potassium ions, from 95.6%±1.7% (25 mM K⁺) and 65.8%±1.9% for (40 mM K⁺), (p<0.05). SNP showed the highest potency in the experiments with PhE and potassium. Although the differences in contractile force were larger, the decrease of potency and efficacy was less marked when compared to the U-46 experiments.

MCh (Figure 4b) responses also displayed a decrease of potency for the two highest concentrations potassium ions from 7.4±0.3 to 6.1±0.1 (for both concentrations, p<0.05). Again the efficacy was only reduced after pre-contraction with 40 mM potassium, from 55.4%±4.6% to 24.5%±0.8% (p<0.05). Similarly as in the SNP/K⁺ experiments notwithstanding the more prominent increase in contractile force, the potency and efficacy of MCh in the potassium experiments are less pronounced, when compared to the experiments MCh/U-46.

An increase in [K⁺] resulted in a decrease of the vasodilator potency of FSK (Figure 4c). The potency decreased from 7.1±0.0 to 6.6±0.0 (p<0.05) and further to 5.8±0.1 (p<0.05) for the potassium ion concentrations of 25 mM, 30 mM and 40 mM, respectively. When compared with PhE and U-46 the decreases in potency after K⁺ pre-contraction proved more pronounced. Accordingly, the slopes of the curves of FSK after PhE and potassium are steeper when compared with the FSK curve after pre-contraction with U-46.

The levels of pre-contraction (Figure 1) by PhE and K⁺ -ions increased when repeated in the continuous presence of L-NAME 100 μM, that is from 4.87 mN ± 0.34 mN to 11.49 mN ± 0.89 mN (p<0.05) and from 7.54 mN ± 0.53 mN to 14.06 mN ± 0.74 mN (p<0.05) for 0.1 μM and 3 μM PhE, respectively. The potassium concentrations of 25 and 40 mM showed comparable increases in contractile force of 4.01 mN ± 0.42 mN to 8.40 mN ± 0.99 mN (p<0.05) and from 10.77 mN ± 0.38 mN to 13.51 mN ± 1.65 mN (p<0.05), respectively, when established in the presence of L-NAME 100 μM. The observed potencies in the presence of L-NAME showed only a tendency to increased after pre-contraction with PhE at 0.1 and 3 μM when compared with the experiments in the absence of L-NAME (Figure 2a and 4a). This tendency occurred in spite of
differences in contractile force generated in both in the presence and absence of L-NAME, respectively.

The CRC for nifedipine were virtually the same when constructed in the presence of the various concentrations of St 587 investigated. Data are not shown.

4. Discussion

Although the effect of vasodilator drugs can be investigated qualitatively and quantitatively at the levels of membrane currents and potential, calcium sequestration, and second messenger formation, their effect on the function of the integrated system of a whole vessel preparation remains the gold standard in in-vitro pharmacological research. However, there are certain prerequisites and limitations to this experimental approach. Besides the differences between species, age and sex, the observation of differences in sensitivity of various vascular beds to the endogenous and exogenous vasoconstrictor and dilator substances has been extensively described in the literature.

A second important point is the very nature of a vasodilatory drug that it exerts a functional effect in the presence of smooth muscle activity only. So the consequences of the interaction of these compounds with their target structure, e.g. receptors coupled to a NO-synthase, second messenger generating systems or ion channels, become only visible if there is an active muscular tone to be counteracted. This in its turn implies that the degree of and/or mechanism behind a contraction, i.e. the pre-contraction, present at the time of exposition to the dilator drug may influence its pharmacological characteristics in terms of potency and/or efficacy. It was the aim of the present study to test this hypothesis by means of applying various pre-contracting compounds in different concentrations followed by cumulative concentration response curves for the various vasodilators.

Phenylephrine (PhE) is a non-subtype selective agonist of $\alpha_1$-adrenoceptors, that belong to the superfamily of G-protein-coupled receptors. The interaction of PhE with $\alpha_1$-adrenoceptors on vascular smooth muscle results in the activation of at least two different signal transduction cascades: the phospholipase C-induced phosphoinositol breakdown with a subsequent release
of calcium from intracellular stores and the activation of calcium channels in the outer plasma membrane leading to a calcium influx. This is in contrast with the effect of the imidazolidine derivative St 587, another, although partial, $\alpha_1$-adrenoceptor agonist, that is mainly dependent on calcium influx via nifedipine-sensitive L-type calcium channels. Binding of the thromboxane $A_2$-like agonist U-46616 (U-46) to its receptors on the surface vascular smooth muscle cells, activates the phospholipase C pathway. Its contractile effect is thereby mainly independent from the extra-cellular calcium concentration.

Increasing the extra cellular potassium concentration above 10 mM results in a concentration-dependent membrane-depolarisation with a concomitant opening of voltage-gated calcium channels and an influx of this divalent ion. This type of response, which is sensitive to the L-type calcium-channel blocker nifedipine, is completely dependent on the extracellular calcium-concentration.

Methacholine (MCh) is a metabolically stable derivative of the transmitter acetylcholine. Like acetylcholine, methacholine activates all subtypes of muscarinic receptors. However, the MCh-induced vasodilation is mainly due to the stimulation of endothelial $M_3$-type muscarinic receptors. These receptors are coupled to the endothelial nitric oxide synthase. On stimulation, this enzyme catalyses the formation of nitric oxide and citrulline, using L-arginine as a substrate. NO diffuses to the underlying smooth muscle layer, enters into the cells and activates the soluble form of guanylate cyclase, thus resulting in the formation of cGMP. This second messenger is responsible for the relaxation due to reduction of the free intracellular $\text{Ca}^{2+}$ concentration and desensitisation of the contractile apparatus to $\text{Ca}^{2+}$ probably by activating myosin light chain phosphatase. The effect of muscarinic agonists like methacholine is therefore completely dependent on the presence of a functional endothelium. This is in contrast to sodium nitroprusside (SNP), which is an endothelium-independent NO-donor that induces the same cascade of events in smooth muscle cells as endothelium-derived nitric oxide. Forskolin (FSK), an alkaloid from Coleus forskholii, is a selective, cell permeable activator of adenylate cyclase, an enzyme that catalyses the formation of cyclic adenosine monophosphate (cAMP). This second messenger induces relaxation by a protein kinase A-dependent increase of calcium efflux and uptake by the endoplasmatic reticulum as well as a reduction in the myosin light chain kinase sensitivity to calcium ions.
The chosen concentrations of the vasoconstrictors, with the exception of St 587, resulted in different degrees of contraction, a prerequisite for the main objective of the present study. Increasing vascular smooth muscle tone reduced the potency of the cGMP-dependent vasodilators SNP and MCh, independent of the contractile stimulus used. The potency of the cAMP-dependent vasodilator FSK, on the other hand, appeared to be uninfluenced by the degree of PhE induced precontraction, but it was reduced when concentrations of U-46 or K^+ ions were increased.

The efficacy (E_{max}) of the endothelium-dependent, NO-releasing vasodilator methacholine was decreased with increasing concentrations of all three of the contractile stimuli, whereas the NO-donor SNP remained fully effective at all PhE-concentrations but was found to be submaximally effective at higher concentrations of U-46 and K^+ ions. It draws the attention that the efficacy (E_{max}) of FSK was unchanged independent of the type of vasoconstrictor or the degree of smooth muscle tension.

Although the increasing concentrations of the three contractile stimuli did not produce identical absolute forces, a compound-specific impact on the vasodilators efficacy and potency can be derived from differential effects of equieffective vasoconstrictive concentrations. Evaluating the results of the FSK experiments the slopes of U-46 are significantly decreased compared to the experiments with K^+ ions. This finding suggests that, after K^+ precontraction, there occurs a monophasic response of the voltage gated Ca^{2+} channels to the rise of [c-AMP]. The decreased slope of the FSK concentration relaxation curve suggests an inhibitory activity of U-46. Probably mediated by the β isoform of the thromboxane A2 receptor, that is known to interact with adenylate cyclase activation, as has been described for internal mammary artery preparations.

In general it can be stated that the vasodilators that exert their effect via the NO-dependent activation of soluble guanylate cyclase, either as a NO-donor (SNP), or as an activator of the endothelial NO-synthase (MCh) are much more influenced by the characteristics of the precontraction than FSK, a vasodilator operating via cAMP. This difference between vasodilators involving cGMP and cAMP, respectively, was observed irrespective of the type of vasoconstrictor used for precontraction. Although this phenomenon needs further
investigation, it can be speculated that quantitative and/or qualitative effects might be responsible for the observed differences. Vasodilators may display different efficacies due to their quantitative ability to induce the production of cAMP or cGMP, respectively. These second messengers in their turn might display important differences regarding their efficacy at the target structures like proteins kinases A and G (PKA and PKG), or the cyclic nucleotide-gated channels, or, even more downstream, at the level of IP3 inhibition. The complexity of these second messenger cascades is even increased by the fact that cAMP cross-activates PKG in the rat aorta, and this phenomenon probably holds true for cGMP activating PKA as has been shown in human intestinal cells.

Another explanation for the observed differences in vasodilating properties between cAMP and cGMP dependent vasodilators may be the pre-experimental activation of these second messenger systems. In a comparable to our experimental in vitro set-up, measurements of cGMP formation demonstrate a low basal level, under the continuous influence of spontaneous NO release. Additionally there is extensive evidence that vasoconstrictors can amplify the NO-release from the vascular endothelium.

This implicates that there is a certain amount of NO present before the administration of the vasodilators, which is possibly dependent on the type and concentration of the vasoconstrictor and the resulting degree of vasoconstriction. Consequently, soluble guanylate cyclase and its subsequent signal transduction cascade is at least partially activated. Under these conditions, an additional activation of this system by NO-donors like SNP or endothelium-dependent NO-releasing agents like MCh might be less effective compared to the cAMP-dependent vasodilator forskolin. Consequently the more apparent loss of efficacy and/or potency of cGMP-mediated vasodilators could be due to an agonist-induced NO-release as the basis for the loss in potency and/or efficacy of these vasodilators.

To evaluate to which extent this effect may have influenced the results, additional experiments with SNP after precontraction with either potassium or PhE were performed in the presence of the NO-synthesis inhibitor L-NAME (figure 2a and 4a). As expected NO-synthesis inhibition increased the contractile efficacy of both, potassium and PhE (figure 1). This indicates at
least a spontaneous NO-release, probable amplified by the PhE- and potassium induced vasoconstrictor effect. However, the reduction of the potency of SNP by about one order of magnitude, when the concentration of the precontracting stimuli was increased, was unmodified by L-NAME, suggesting that endogenous NO had any influence on the potency shift.

To further test whether there is an adrenoceptor-specific mechanism involved, the partial $\alpha_1$-agonist St 587 was used. The signal-transduction cascade of this atypical compound does not contain second-messenger systems and can be selectively and quantitatively blocked by L-type calcium channel blockers such as nifedipine. The vasodilator potency and efficacy of nifedipine was independent of the St 587 concentration used. This supports the view that especially second messenger-mediated vasodilating effects are susceptible to the degree of precontraction and the type of stimulus by which it is induced.

Precontraction by increasing concentrations of the thromboxane A$_2$ agonist resulted most clearly in a decrease in potency and efficacy of the cGMP-mediated vasodilators, whereas gradual depolarisation by increasing concentrations of potassium ions resulted in the greatest decrease of potency of the cAMP-mediated vasodilator FSK. Although numerous mechanisms underlying vasodilation and contraction have been discovered in the recent years, reliable predictions to which degree a vasoconstrictor used for precontraction will influence the properties of vasodilators could not be made, yet.

From the presented data it is concluded that the degree of pre-contraction and the type of stimulus by which it is induced markedly influence the pharmacological characteristics of second-messenger-dependent vasodilators. Cyclic GMP-mediated vasodilator responses are more sensitive in this respect than mechanisms involving cyclic AMP. Studies, comparing the pharmacological characteristics of different vasodilators should therefore be performed only under identical conditions, using the same stimulus, preferably at a submaximal effective concentration.
5. References

Vasodilator drugs and pre-contraction in vitro


23. Chao AC, De Sauvage FJ, Dong YJ, Wagner JA, Goddel DV, Gardner P. Activation of intestinal CPT Ccl channel by heat-stable enterotoxin and guanylin via CAMP.


