ELSEVIER

European Journal of Pharmacology 437 (2002) 155–163

www.elsevier.com/locate/ejphar

Interactions of sildenafil with various coronary vasodilators in isolated porcine coronary artery

Ichiro Sakuma [a], Yasuhiro Akaishi [b], Hiroshi Tomioka [a], Atsushi Sato [b], Akira Kitabatake [a], Yuichi Hattori [b,*]

[a] *Department of Cardiovascular Medicine, Hokkaido University School of Medicine, Sapporo 060-8638, Japan*
[b] *Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060-8638, Japan*

Received 3 September 2001; received in revised form 21 November 2001; accepted 24 December 2001

Abstract

There are reports of serious hypotension or circulatory shock when sildenafil citrate, a selective cyclic nucleotide phosphodiesterase type 5 inhibitor, which was developed for the treatment of erectile dysfunction, is given to patients taking certain coronary vasodilators. We thus examined the interaction of sildenafil with various coronary vasodilators including nitric oxide (NO) donors in isolated porcine coronary artery. Sildenafil caused concentration-dependent relaxations of the artery precontracted with U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$). Incubation with the NO synthase inhibitor N^G-nitro-L-arginine or the soluble guanylate cyclase inhibitor ODQ (1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one) significantly shifted the concentration–response curve for sildenafil to the right without affecting the maximum response, indicating that some part of the relaxant response to sildenafil may be the result of the inhibition of phosphodiestrase type 5-induced degradation of cyclic GMP (cGMP) that is produced through guanylate cyclase activation by NO released spontaneously. The relaxant effects of the vasodilators with an NO donor property, isosorbide dinitrate, sodium nitroprusside, nicorandil and nipradilol, were significantly enhanced by sildenafil, as shown by a significant leftward shift of their concentration–response curves. In contrast, the relaxant responses to the drugs without a property as an NO donor, diltiazem, celiprolol and pinacidil, were not affected by sildenafil. The cGMP level of the tissue was elevated after adding sildenafil, and the cGMP-generating effect of a combination of sildenafil and sodium nitroprusside was higher than that of each drug alone. The cyclic AMP level determined simultaneously was not changed by sildenafil. These results suggest that sildenafil potentiates specifically the relaxant responses of porcine coronary artery to the drugs which behave as an NO donor, providing basic evidence that the benefit of sildenafil in the treatment of erectile dysfunction can be limited by a risk of marked vasodilation when used together with NO-related coronary vasodilators.

Keywords: Sildenafil; Nitric oxide (NO) donor; Non-nitric oxide (NO) donor; Vasodilation; cGMP; Coronary artery, porcine

1. Introduction

Nitric oxide (NO), through activation of soluble guanylate cyclase, induces the formation of intracellular cyclic GMP (cGMP) from GTP to elicit relaxation of smooth muscle cells (Moncada et al., 1991; Umans and Levi, 1995). Upon penile erection, NO is released from nonadrenergic-noncholinergic neurons and vascular endothelial cells, and cGMP content is elevated in the corpora cavernosa. The accumulated cGMP in turn leads to relaxation of vascular and trabecular smooth muscle of the penis, and the resultant increase in blood flow and engorgement of the trabecular spaces result in pinile erection (Boolell et al., 1996; Burnett, 1997; Ballard et al., 1998).

Sildenafil (Viagra™) is now popularly used for the treatment of patients with erectile dysfunction. This agent is a highly selective inhibitor of phosphodiesterase type 5 (Ballard et al., 1998), the predominant isozyme responsible for the degradation of cGMP in the corpus cavernosum (Boolell et al., 1996; Moreland et al., 1998). Selective inhibition by sildenafil of phosphodiesterase type 5 accelerates NO-induced accumulation of cGMP during penile erection, leading to the enhancement of relaxation of both vascular and trabecular smooth muscle in the corpus cavernosum (Waldman and Murad, 1987; Jeremy et al., 1997). This mode of action suggests that sildenafil may synergistically interact with NO donors, which cause potent vasodilation. Indeed, clinical data have shown that severe hypotension or circulatory shock

* Corresponding author. Tel.: +81-11-706-6920; fax: +81-11-706-7824.
E-mail address: yhattori@med.hokudai.ac.jp (Y. Hattori).

0014-2999/02/$ - see front matter
PII: S0014-2999(01)01622-3

often occurs when sildenafil is given to the patients taking NO-related coronary vasodilators (Webb et al., 1999; Zusman et al., 1999; Kloner, 2000). To our knowledge, however, only a few studies using isolated arteries have been done to provide basic evidence for the interaction of sildenafil or other phosphodiesterase type 5 inhibitors and NO donors (Medina et al., 2000; Takagi et al., 2001). Furthermore, the interactions of sildenafil and other coronary vasodilators as non-NO donors remain unknown.

The present study was undertaken to examine how sildenafil can modify the relaxant effects of various coronary vasodilators, including NO donors, Ca^{2+} channel blocker, K^+ channel openers and β-adrenoceptor-acting agents, which can be currently available for the treatment of coronary heart disease, in isolated porcine coronary artery. We also investigated the effect of sildenafil on the changes in cGMP and cyclic AMP (cAMP) contents caused by some vasodilators in this tissue to determine whether sildenafil has a synergistic action on the relaxant responses to the vasodilators in relation to its effect on cyclic nucleotide metabolism.

2. Materials and methods

2.1. Tension measurement

Porcine hearts were obtained from a slaughterhouse and transported in ice-cold oxygenated physiological salt solution (PSS). The composition of PSS was as follows (in mM): NaCl 118.2, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0. The left circumflex coronary artery (outer diameter, ~2 mm) was dissected from the heart in oxygenated PSS. The isolated artery was trimmed of fat and connective tissues under a dissecting microscope and cut into rings 4 mm in length. Care was taken to ensure that the endothelium was not damaged during the processing of the tissue preparation. Where indicated, the endothelial cells were removed by gently rubbing the intimal surface of the vessel with a moistened cotton ball. The arterial ring was suspended by a pair of stainless steel hooks in a water-jacketed bath filled with 25 ml of PSS. The solution in the bath was gassed with 95% O_2 and 5% CO_2, and its temperature was maintained at 37 °C. The ring was stretched until an optimal tension of 2 g was loaded and then allowed to equilibrate for at least 60 min before the start of the recordings. Isomeric tension was monitored with a transducer and recorded by a pen recorder. The rings were repeatedly challenged with 40 mM K^+ until the high K^+-induced contractions reached a constant value. High K^+ PSS was prepared by substitution of KCl for NaCl on an equimolar basis.

The relaxant responses to various vasodilators were examined in the absence and presence of 1 μM sildenafil during the contractions induced by 100 nM U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$), a stable analogue of thromboxane A_2. Each vasodilator was cumulatively added into the organ bath after the contraction by U46619 had reached a plateau level. In the experiment with sildenafil, the addition of each vasodilator was started after a steady effect of sildenafil had been obtained. The relaxant responses to the vasodilator with and without sildenafil were always run in parallel experiments. In a series of experiments carried out to examine the involvement of endothelium-derived NO or soluble guanylate cyclase in the direct relaxant effect of sildenafil, 100 μM N^G-nitro-L-arginine (L-NNA) or 10 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one) was added to the bath 20 min before application of U46619, and then sidenafil was added in a cumulative manner during U46619-induced contractions. The degree of relaxations was expressed as a percentage of the height of contraction induced by U46619. In a series of experiments carried out to examine the concentration–response curves for coronary vasodilators in the absence and presence of 1 μM sildenafil, relaxations were also expressed as a percentage of the contraction level when sildenafil-induced relaxation was subtracted from the total relaxation.

2.2. Cyclic nucleotide assays

The coronary arterial rings were mounted in the organ bath in the same way as described above. The rings were removed from the organ bath at 5 min after the addition of each vasodilator unless otherwise specified, and were immediately frozen with a clamp which had been cooled with liquid nitrogen. The frozen tissues were weighed and homogenized in 1 ml of ice-cold 6% trichloroacetic acid. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatant was reserved. The pellet was resuspended in 1 ml of cold 6% trichloroacetic acid and centrifuged again. After the addition of 10 μl of 1 N HCl, the

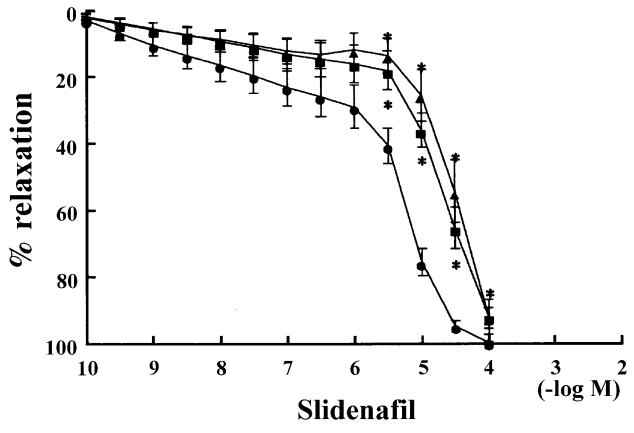

Fig. 1. Concentration–response curves for the relaxant effect of sildenafil in porcine coronary artery precontracted with 100 nM U46619. The curve for sildenafil in the absence of any treatment (●) is compared with those in the presence of 100 μM L-NNA (■) or 10 μM ODQ (▲). The responses are expressed as percent relaxation of U46619-induced contraction. Points are mean ± S.E. of nine experiments. *$P < 0.05$ vs. the corresponding values in the absence of any treatment.

combined supernatant fractions were extracted 4 times with 1 ml of water-saturated ether, heated at 80 °C for 3 min to evaporate the residual ether, then lyophilized overnight and resuspended in distilled water. The cGMP and cAMP contents were determined by the sensitive radioimmunoassay method described by Honma et al. (1977) using a YAMASA cGMP and cAMP kit (Yamasa Shoyu, Choshi, Japan).

2.3. Statistical analysis

All values were expressed as mean ± S.E.M. Statistical assessment of the data was made by Student's *t*-test or one-way ANOVA (analysis of variance) followed by the Fisher's post hoc test when appropriate. A P value < 0.05 was considered statistically significant.

2.4. Drugs

The following compounds were used: sildenafil citrate (Pfizer, New York, NY, USA), U46619 (Cayman, Ann Arbor, MI, USA), isosorbide dinitrate (Sigma, St. Louis, MO, USA), sodium nitroprusside (Sigma), diltiazem hydrochloride (Tanabe, Osaka, Japan), pinacidil (Shionogi, Osaka, Japan), nicorandil (Chugai, Tokyo, Japan), celiprolol hydrochloride (Nihon Shin-yaku, Kyoto, Japan), nipradilol (Kowa, Tokyo, Japan), L-NNA (Sigma) and ODQ (Calbiochem-Novabiochem, La Jolla, CA, USA). Sildenafil, nipradilol and ODQ were prepared in dimethyl sulfoxide. Pinacidil, nicorandil and L-NNA were prepared in 0.3 N HCl. Other compounds were prepared in distilled water. Further dilutions to the desired concentrations were made with PSS.

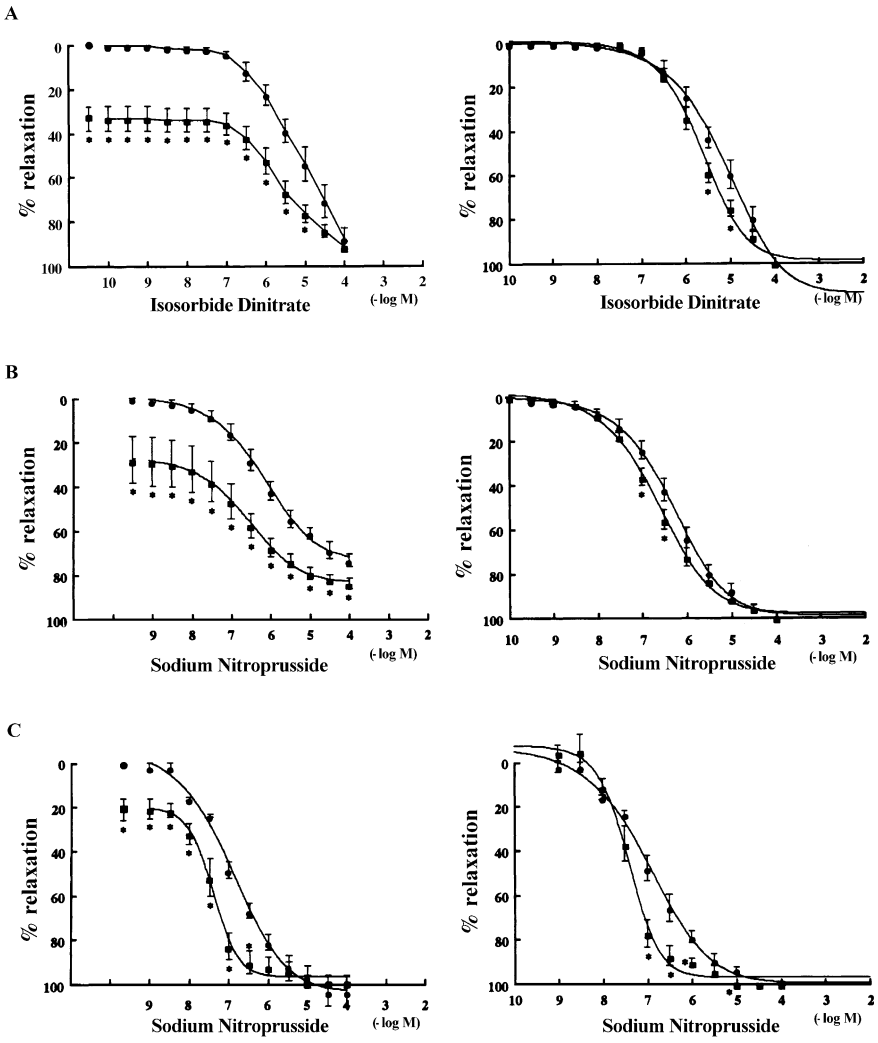

Fig. 2. Influence of sildenafil on the concentration–response curves for the relaxant effects of isosorbide dinitrate (A) and sodium nitroprusside (B, C) in porcine coronary artery precontracted with 100 nM U46619. In each panel are shown the curves in the absence (●) and presence of 1 μM sildenafil (■). In panel C, the preparations where endothelium was mechanically removed were used. Left and right panels show the responses expressed as percent relaxation of U46619-induced contraction and those as percent of the value when the relaxation caused by sildenafil was subtracted from the total relaxation, repectively. Points are mean ± S.E. of four to nine experiments. $*P < 0.05$ vs. the corresponding values in the absence of sildenafil.

3. Results

3.1. Relaxant response to sildenafil

Fig. 1 shows the concentration–response curve for sildenafil-induced relaxation, which was obtained from the experiments where sildenafil was cumulatively given to porcine coronary artery precontracted with 100 nM U46619. Sildenafil revealed a concentration-dependent relaxant effect with a pD_2 value of 5.7 ± 0.2 ($n=9$). Preincubation of the artery with maximally effective concentration of the NO synthase inhibitor L-NNA (100 μM) significantly shifted the concentration curve for the relaxant effect of sildenafil to the right, although the maximum response to sildenafil was unchanged. Thus, the pD_2 value was decreased to 4.8 ± 0.1 ($n=9$, $P<0.001$) in the presence of 100 μM L-NNA. The same rightward shift of the concentration curve for the relaxant effect of sildenafil occurred in the presence of the soluble guanylate cyclase inhibitor ODQ (10 μM) ($pD_2 = 4.7 \pm 0.1$, $n=9$, $P<0.001$).

3.2. Influence of sildenafil on relaxant responses to various vasodilators

In order to elucidate the effect of sildenafil on the relaxant responses to the vasodilators with a pure NO donor property, isosorbide dinitrate and sodium nitroprusside, the concentration–response curves for these vasodilators were determined in the absence and presence of sildenafil; the results are shown in Fig. 2A and B. The concentration–response

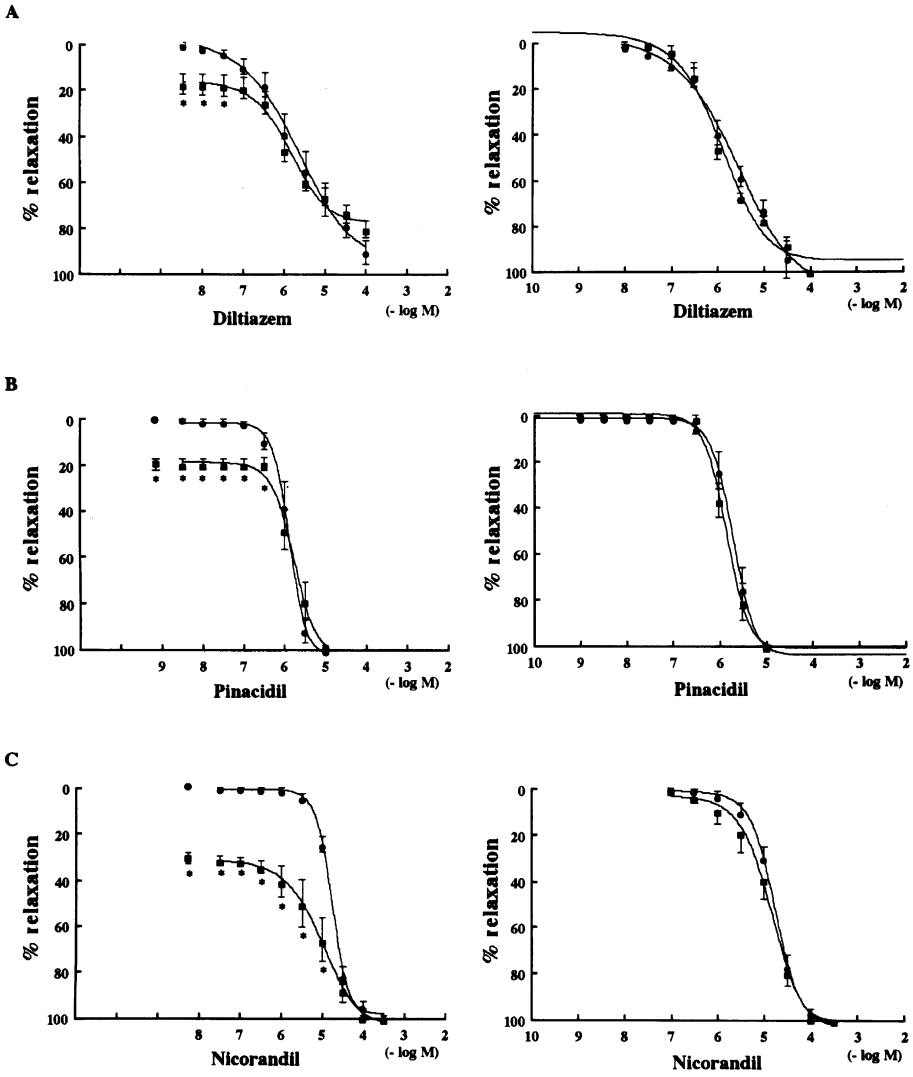

Fig. 3. Influence of sildenafil on the concentration–response curves for the relaxant effects of diltiazem (A), pinacidil (B) and nicorandil (C) in porcine coronary artery precontracted with 100 nM U46619. In each panel are shown the curves in the absence (●) and presence of 1 μM sildenafil (■). Left and right panels show the responses expressed as percent relaxation of U46619-induced contraction and those as percent of the value when the relaxation caused by sildenafil was subtracted from the total relaxation, repectively. Points are mean ± S.E. of six to nine experiments. *$P<0.05$ vs. the corresponding values in the absence of sildenafil.

curves for isosorbide dinitrate and sodium nitroprusside were shifted to the left and downward in the presence of 1 μM sildenafil. In other words, the relaxant responses to isosorbide dinitrate and sodium nitroprusside were enhanced by 1 μM sildenafil. When the relaxation caused by sildenafil was subtracted from the total relaxation, and the U46619-induced contraction level recorded before application of isosorbide dinitrate or sodium nitroprusside was taken as 100%, the concentration–response curves for isosorbide dinitrate and sodium nitroprusside appeared to be shifted to the left in a parallel manner (right panels in Fig. 2). The pD_2 values for isosorbide dinitrate and sodium nitroprusside calculated in such a way were significantly higher in the presence of sildenafil. Thus, the values for isosorbide dinitrate were 5.3 ± 0.1 ($n=6$) and 5.7 ± 0.1 ($n=6$, $P<0.05$), and those for sodium nitroprusside were 6.3 ± 0.1 ($n=9$) and 6.7 ± 0.1 ($n=9$, $P<0.05$) in the absence and presence of 1 μM sildenafil, respectively. Removal of the endothelium partially inhibited the relaxation induced by sildenafil alone as pretreatment with L-NNA did; the relaxant effect of 1 μM sildenafil tended to be reduced from $28 \pm 11\%$ ($n=9$) to $17 \pm 5\%$ ($n=6$) by endothelium removal. However, the enhancing effect of sildenafil on the SNP-induced relaxation was substantially unchanged in endothelium-denuded artery (Fig. 2C).

As shown in Fig. 3A, the Ca^{2+} channel blocker diltiazem produced relaxation in a concentration-dependent manner. The cumulative concentration–response curve for diltiazem constructed in the presence of 1 μM sildenafil was not significantly different from the curve obtained in its absence. The pD_2 values for diltiazem were 5.8 ± 0.1 ($n=9$) and 5.9 ± 0.1 ($n=9$, $P=0.49$) in the absence and presence of 1 μM sildenafil, respectively.

The pure K^+ channel opener pinacidil produced a concentration-dependent relaxant effect with a pD_2 value of 5.8 ± 0.1 ($n=6$) (Fig. 3B). Sildenafil marginally affected the relaxant effect of pinacidil, as judged from the lack of substantial shift of the concentration–response curve for pinacidil in the presence of 1 μM sildenafil. The pD_2 value for pinacidil in the presence of sildenafil was 5.9 ± 0.1 ($n=6$, $P=0.46$). In contrast, the cumulative concentration–response curve for nicorandil, which has a hybrid property between nitrates and K^+ channel openers, was shifted to the left by 1 μM sildenafil in a somewhat nonparallel fashion (Fig. 3C). Thus, sildenafil potentiated the responses to lower concentrations (≤ 3 μM) of nicorandil. The relaxant effect of 3 μM nicorandil was $5 \pm 1\%$ ($n=7$) in the absence and $12 \pm 3\%$ ($n=7$, $P<0.05$) in the presence of 1 μM sildenafil.

The influence of sildenafil on the relaxant effects of two β-adrenoceptor-acting agents, celiprolol and nipradilol, were also tested. Celiprolol is a selective β_1-adrenoceptor antagonist with a partial agonistic action on β_2-adrenoceptors, the latter accounting for the large part of its vasodilatory action (Brodde et al., 1986; Dhein et al., 1992). As shown in Fig. 4A, higher concentrations of celiprolol were required to produce a relaxant effect, resulting in its estimated pD_2 of

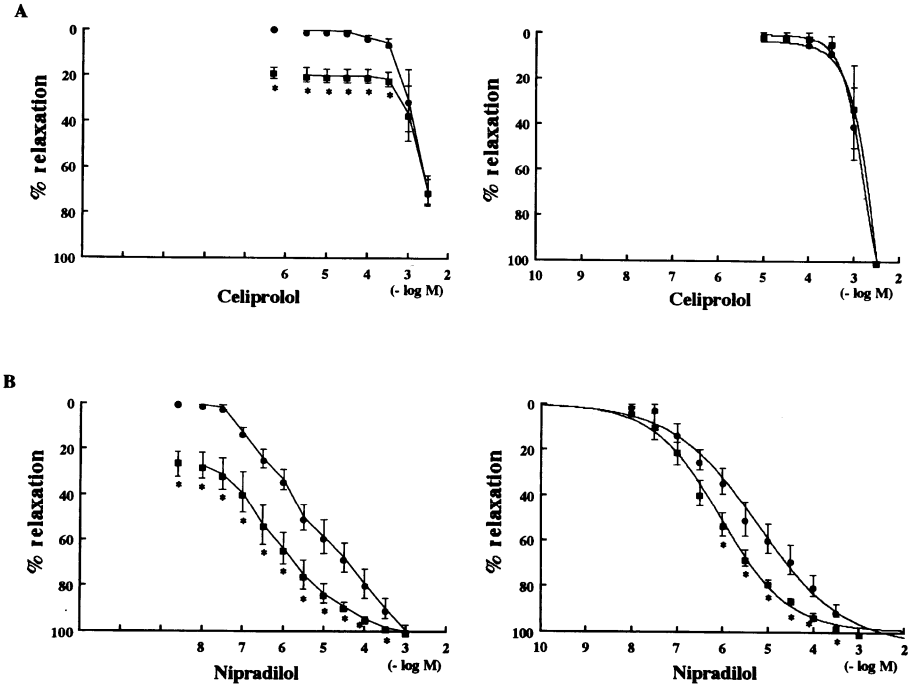

Fig. 4. Influence of sildenafil on the concentration–response curves for the relaxant effect of celiprolol (A) and nipradilol (B) in porcine coronary artery precontracted with 100 nM U46619. In each panel are shown the curves in the absence (●) and presence of 1 μM sildenafil (■). Left and right panels show the responses expressed as percent relaxation of U46619-induced contraction and those as percent of the value when the relaxation caused by sildenafil was subtracted from the total relaxation, repectively. Points are mean ± S.E. of five to six experiments. *$P<0.05$ vs. the corresponding values in the absence of sildenafil.

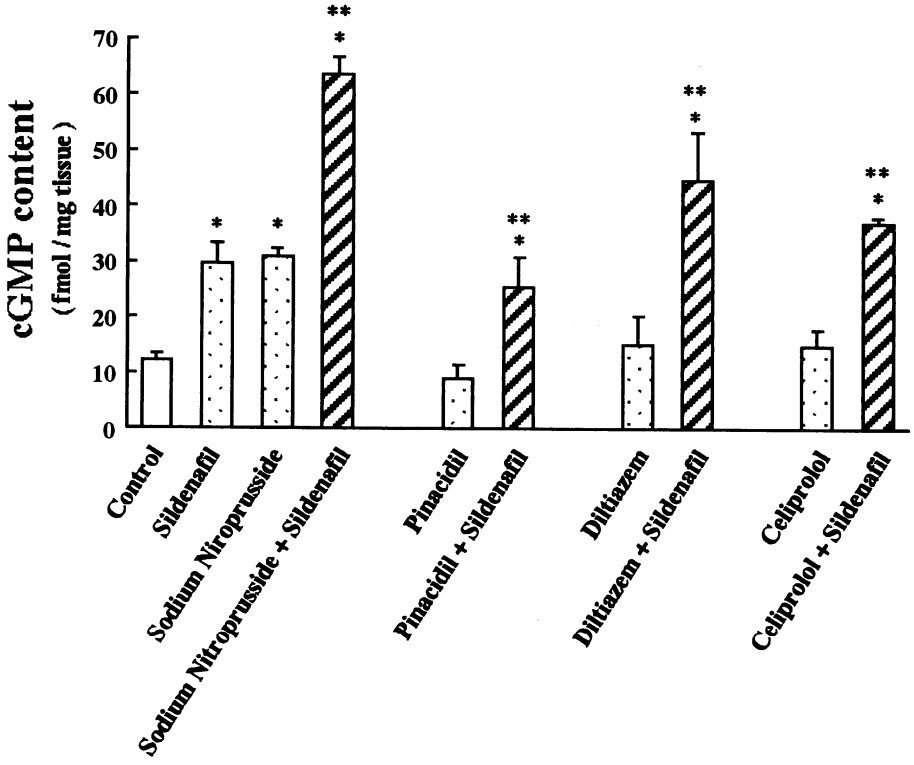

Fig. 5. Influences of sildenafil (1 μM), sodium nitroprusside (100 nM), pinacidil (1 μM), diltiazem (1 μM) and celiprolol (1 mM) on the cGMP levels of porcine coronary artery. Bars represent means ± S.E. of four experiments. *P value indicates that the cGMP level is significantly (<0.05) elevated in comparison with the control value. **P value indicates that the cGMP level in the presence of each vasodilator is significantly (0.05) enhanced by combination with sildenafil.

3.0 ± 0.1 ($n=6$). Sildenafil at a concentration of 1 μM had no effect on the relaxant response to celiprolol, with no substantial shift of the concentration–response curve for celiprolol. The pD_2 value for celiprolol in the presence of 1 μM sildenafil (2.9 ± 0.1, $n=6$, $P=0.50$) was almost the same as that in its absence. Nipradilol is a nonselective β-

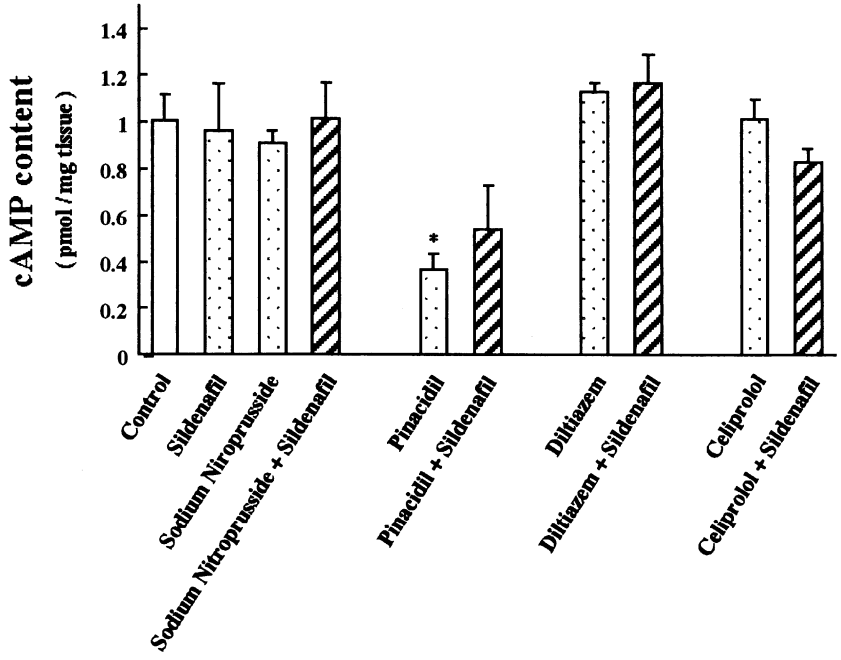

Fig. 6. Influences of sildenafil (1 μM), sodium nitroprusside (100 nM), pinacidil (1 μM), diltiazem (1 μM) and celiprolol (1 mM) on the cAMP levels of porcine coronary artery. Bars represent means ± S.E. of four experiments. *P value indicates that the cAMP level is significantly (<0.05) reduced in comparison with the control value.

adrenoceptor antagonist with vasodilating properties. The vasodilatory effect of nipradilol is based on its action as an NO donor and a weak α-adrenoceptor antagonist (Abe et al., 1996; Hayashi and Iguchi, 1998). In contrast to the effect of celiprolol, the relaxant effect of nipradilol was significantly potentiated by 1 μM sildenafil. The concentration–response curve for nipradilol was evidently shifted to the left in the presence of sildenafil (Fig. 4B). The pD_2 value for nipradilol was significantly increased from 5.3 ± 0.2 ($n = 5$) to 6.1 ± 0.1 ($n = 5$, $P < 0.05$) by sinadilfil.

3.3. Effects of sildenafil and various vasodilators on cyclic nucleotide levels

As shown in Fig. 5, the cGMP level of the tissue was elevated after adding 1 μM sildenafil; it reached a level about threefold higher than the control level. The increase in the cGMP level caused by sodium nitroprusside at a concentration of 100 nM, which is almost equal to the concentration producing about 30–40% relaxation of coronary artery, was the same as that by 1 μM sildenafil. The cGMP level was elevated more in the presence of both sildenafil and sodium nitroprusside than with each drug alone. The cGMP level was not affected significantly by the drugs without a property as an NO donor, pinacidil, diltiazem and celiprolol at the concentrations necessary to relax the artery about 30–40% of their own maximum relaxation (1 μM, 1 μM and 1 mM, respectively). In the presence of these drugs, the extent of the sildenafil-induced increase in the cGMP level was not changed.

As shown in Fig. 6, the cAMP level was not changed significantly after the addition of 1 μM sildenafil. None of sodium nitroprusside, diltiazem and celiprolol did affect the cAMP level regardless of whether sildenafil was present. Pinacidil caused a significant reduction in the cAMP level. The reduced cAMP level was not reversed by sildenafil.

4. Discussion

In this study, we demonstrated that sildenafil by itself produced a concentration-dependent relaxation of porcine coronary artery. Incubation with the NO synthase inhibitor L-NNA caused a decrease in the sensitivity to sildenafil. A similar rightward shift of the sildenafil concentration–response curve was observed in the presence of the soluble guanylate cyclase inhibitor ODQ. In view of strong evidence that sildenafil is a highly selective inhibitor of phosphodiesterase type 5 that is responsible for the degradation of cGMP (Ballard et al., 1998), these findings imply that sildenafil accelerates cGMP accumulation, which is induced by NO released spontaneously via stimulation of soluble guanylate cyclase, thereby resulting in vascular relaxation. Indeed, the relaxant effect of sildenafil was accompanied by a concomitant elevation of the cGMP level of the tissue. However, the fact that there was no change in the maximum response to sildenafil in the presence of maximally effective concentrations of L-NNA and ODQ can be interpreted to reflect that these inhibitors only partially inhibited the relaxant effect of sildenafil. Thus, the residual relaxation to sildenafil in the presence of L-NNA or ODQ indicates that the large part of the vasorelaxant action of sildenafil occurs independently of the NO-soluble guanylate cyclase pathway. This was in contrast with the finding that the relaxant effect of other phosphodiesterase type 5 inhibitor (T-1032) in rat aorta was greatly inhibited by both ODQ and N^G-nitro-L-arginine methyl ester (Takagi et al., 2001). Recent report suggests that sildenafil at concentrations of ~30 μM directly blocks ionic membrane currents such as the delayed rectifier K^+ current in cardiac myocytes (Geelen et al., 2000). It is possible that sildenafil may cause a block of several ionic channels in vascular smooth muscle cells. We speculate that the vasorelaxant effect of higher concentrations of sildenafil may be the result of direct electrophysiological actions such as blockade of L-type Ca^{2+} channels, independently of cyclic nucleotide metabolism. Relaxations to sildenafil at the highest concentration (30 μM) have been reported to be only about 50% in coronary artery but complete in mammary and radial arteries isolated from human (Medina et al., 2000). Thus, the endothelium-independent relaxant effect of sildenafil that occurred at the high concentrations may differ by species and/or anatomic origin of the artery.

Because 1 μM sildenafil alone caused modest relaxation, when the relaxant effects of a series of coronary vasodilators were tested in the absence and presence of 1 μM sildenafil, we considered the concept for the estimation of combined drug effects by means of the construction of the theoretical concentration–response curves (Pöch et al., 1990, 1995; Holzman et al., 1992). Then, a shift of the relative concentration–response curves for the net effects of coronary vasodilators to the left to higher pD_2 values by sildenafil should allude to potentiation.

In the presence of 1 μM sildenafil, the relaxant effects of isosorbide dinitrate, sodium nitroprusside and nipradilol, all of which have an NO donor property, were enhanced. A significant increase in the sensitivity to these NO donors was found in the presence of sildenafil, as assessed by their pD_2 values. The enhancement of the vasorelaxant response to nicorandil by sildenafil was evident only at lower concentrations of nicorandil. The action as a K^+ channel opener occurs with the concentrations of nicorandil that exceed those which elevate cGMP of vascular tissues (Weir and Weston, 1986; Miyata et al., 1990), suggesting that nicorandil behaves predominantly as an NO donor at lower concentrations, whereas hyperpolarization-mediated relaxation prevails at higher concentrations. It is thus most likely that sildenafil enhanced the relaxant response to nicorandil, which is based on the action as an NO donor. The concentration of sildenafil employed in this study (1 μM) is equivalent to the peak plasma concentration that is clinically achievable (technical report from Pfizer). Furthermore, it has to be taken into account that UK-103,320, a main

metabolite of sidenafil, may mimic the action of sildenafil, because this metabolite is structurally quite similar to the parent drug having a pyrazol ring. In fact, UK-103,320 serves as a phosphodiesterase type 5 inhibitor that is two fifth as potent as sildenafil (technical report from Pfizer). The plasma concentrations of sildenafil and its metabolites can be elevated around twofolds in the cases of advanced age, with hepatic or renal dysfunction, and so forth (technical report from Pfizer). The plasma level of sildenafil would be also increased in patients with genetic variation or abnormality of cytochrome *P*450 (CYP) enzymes, among which CYP3A4 and CYP2C9 are mainly responsible for the metabolism of sildenafil (Warrington et al., 2000). Thus, we interpret the present findings to indicate that the vasodilating effects of the drugs with an NO donor property are significantly enhanced by sildenafil at the concentration that can be achieved in clinical practice.

In view of the ability of sildenafil to inhibit cGMP hydrolysis by phosphodiesterase type 5 (Ballard et al., 1998), the effectiveness of sildenafil in enhancing the vasorelaxant responses to NO donors should be associated with an increase in the cGMP levels. Thus, the potency of NO donors to relax the vascular smooth muscle would be increased presumably due to functional inhibition of phosphodiesterase type 5 by sildenafil, which prevents the breakdown of cGMP. The increase in cGMP caused by sildenafil alone, which was considered to be the result of the enhancement of tonic stimulation of guanylate cyclase with endothelium-derived intrinsic NO, is less likely to promote the enhancement of the vasorelaxant responses to NO donors, since we observed that sildenafil enhanced the relaxant effect of sodium nitroprusside regardless of whether the endothelium was present. The cGMP level was elevated more in the presence of both sildenafil and sodium nitroprusside than with each drug alone. However, no assertion about additivity or potentiation could drawn from these findings.

We found that sildenafil did not substantially affect the relaxant responses to the coronary vasodilators that have no property as an NO donor, the Ca^{2+} channel blocker diltiazem and the K^+ channel opener pinacidil. In addition, sildenafil was without effect on the relaxant response to celiprolol. Although celiprolol has been reported to produce vasodilation through its partial agonistic action on β_2-adrenoceptors (Brodde et al., 1986; Dhein et al., 1992), celiprolol failed to cause a significant increase in the cAMP level of the tissue. The lack of effect on cAMP may be explained by the fact that coronary β-adrenoceptors in pigs are predominantly of β_1-subtype (Nishimura et al., 1987). In other words, some unknown mechanism other than β_2-adrenoceptor stimulation may be involved in mediating relaxation of porcine coronary artery in light of the higher concentrations of celiprolol required to produce this response. Accordingly, the finding that the vasorelaxant response to celiprolol was unaffected by sildenafil does not exclude the possibility that sildenafil may enhance the vasorelaxant action of cAMP-generating agents. It is well accepted that cGMP inhibits phosphodiestrase type 3, resulting in the enhanced tissue levels of cAMP (Palmer and Moncada, 1989). Several agents that generate cGMP formation have shown to potentiate isoproterenol-induced vascular relaxation by increasing the cAMP level much more than isoproterenol alone, possibly due to inhibition of phosphodiestrase type 3 (Marurice et al., 1991; Satake et al., 1995). However, we found that sildenafil (1 μM) had no effect on the concentration-dependent relaxant response of porcine coronary artery to forskolin, a direct activator of adenylate cyclase (unpublished observation). Furthermore, even if the endothelium was intact, sildenafil failed to increase the basal tissue level of cAMP. Therefore, it seems unlikely that sildenafil may cause a indirect inhibition of phosphodiestrase type 3 by preventing the breakdown of cGMP which is generated by endothelium-derived intrinsic NO, thereby leading to the potentiation of vascular relaxations induced by cAMP-generating agents such as β_2-adrenoceptor agonists through the enhanced cAMP level.

Interestingly, pinacidil significantly reduced the cAMP level of the tissue. It has been reported that a hyperpolarization-activated K^+ efflux appears to directly regulate adenylate cyclase activity in *Paramecium* (Maelicke, 1992; Schultz et al., 1992). There is evidence that activity of membrane-bound enzymes such as phospholipase C can be suppressed by K^+ channel openers through membrane hyperpolarization of vascular smooth muscle (Itoh et al., 1992). Thus, pinacidil may inhibit adenylate cyclase activity possibly due to hyperpolarization of the plasma membrane.

The present study was conducted using isolated porcine coronary artery. It should be kept in mind that the enhancement of NO donor-induced relaxations of porcine coronary artery by sildenafil was evident but not so pronounced. On the other hand, the dramatic drop in mean arterial blood pressure has been observed in patients and experimental animal models when sildenafil and nitrovasodilators are simultaneously given (Webb et al., 1999, 2000). This may be related to fundamental differences between the blood vessels employed in this study and involved in blood pressure. Dilations of resistance and capacitance vessels decrease peripheral resistance and cardiac output, which could in term determine diastolic and systolic arterial blood pressure. It may be inferred that either of these vascular beds reacts more sensitively to the synergistic actions of sildenafil and NO donors.

In conclusion, we showed that sildenafil caused relaxation of porcine coronary artery. This response was partially due to prevention of the phosphodiestrase type 5-induced breakdown of cGMP that is generated by endothelium-derived intrinsic NO. Furthermore, this study is the first to demonstrate that sildenafil, at the concentration that can be achieved in clinical practice, significantly enhanced relaxations induced by the coronary vasodilators behaving as an NO donor. No enhancement of the relaxant responses to non-NO donors, including the Ca^{2+} channel blocker and the K^+ channel opener, was observed. Thus, the present results provide basic evidence for the risk that administration of

sildenafil may easily exert severe hypotension in patients taking NO-related coronary vasodilators.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan, and by Health Sciences Research Grants for Comprehensive Research on Aging and Health from the Ministry of Health and Welfare of Japan.

References

Abe, S., Nakamura, M., Kanaide, H., 1996. Some effects of nipradilol, a β-antagonist possessing a nitroxy group, on smooth muscle of the pig coronary artery. Br. J. Pharmacol. 117, 1707–1715.

Ballard, S.A., Gingell, C.J., Tang, K., Turner, L.A., Price, M.E., Naylor, A.M., 1998. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J. Urol. 159, 2164–2171.

Boolell, M., Allen, M.J., Ballard, S.A., Gep-Attee, S., Muirhead, G.J., Naylor, A.M., Osterloh, I.H., Gingell, C., 1996. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int. J. Impot. Res. 8, 47–52.

Brodde, O.E., Schemuth, R., Brinkmann, M., Wang, X.L., Daul, A., Borchard, U., 1986. β-Adrenoceptor antagonists (non-selective as well as β_1-selective) with partial agonistic activity decrease β_2-adrenoceptor density in human lymphocytes: evidence for a β_2-agonistic component of the partial agonistic activity. Naunyn-Schmiedeberg's Arch. Pharmacol. 333, 130–138.

Burnett, A.L., 1997. Nitric oxide in the pennis: physiology and pathology. J. Urol. 157, 230–234.

Dhein, S., Titzer, S., Wallstein, M., Muller, A., Gerwin, R., Panzner, B., Klaus, W., 1992. Celiprolol exerts microvascular dilatation by activation of β_2-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 27–31.

Geelen, P., Drolet, B., Rail, J., Bérubé, J., Daleau, P., Rousseau, G., Cardinal, R., O'Hara, G.E., Turgeon, J., 2000. Sildenafil (Viagra) prolongs cardiac repolarization by blocking the rapid component of the delayed rectifier potassium current. Circulation 102, 275–277.

Hayashi, T., Iguchi, A., 1998. Nipradilol: a β-adrenoceptor antagonist with nitric oxide releasing action. Cardiovasc. Drug Rev. 16, 212–235.

Holzman, S., Kukovetz, W.R., Braida, C., Pöch, G., 1992. Pharmacological interaction experiments differentiate between glibenclamide-sensitive K^+ channels and cyclic GMP as components of vasodilation by nicorandil. Eur. J. Pharmacol. 215, 1–7.

Honma, M., Satoh, T., Takezawa, J., Ui, M., 1977. An ultrasensitive method for the simultaneous determination of cyclic AMP and cyclic GMP in small-volume samples from blood and tissue. Biochem. Med. 18, 257–273.

Itoh, T., Seki, N., Suzuki, S., Ito, S., Kajikuri, J., Kuriyama, H., 1992. Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. J. Physiol. 451, 307–328.

Jeremy, J.Y., Ballard, S.A., Naylor, A.M., Miller, M.A.W., Angelini, G.D., 1997. Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor, and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum in vitro. Br. J. Urol. 79, 958–963.

Kloner, R.A., 2000. Cardiovascular risk and sildenafil. Am. J. Cardiol. 86 (suppl. 2A), 57F–61F.

Maelicke, A., 1992. An ion channel-gated adenylyl cyclase. Trends Biochem. Sci. 17, 51.

Marurice, D.H., Crankshaw, D., Haslam, R.J., 1991. Synergistic actions of nitrovasodilators and isoproterenol on rat aorta smooth muscles. Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 129–138.

Medina, P., Segarra, G., Martínez-León, J.B., Vila, J.M., Aldasoro, M., Otero, E., Lluch, S., 2000. Relaxation induced by cGMP phosphodiestrase inhibitors sildenafil and zaprinast in human vessels. Ann. Thorac. Surg. 70, 1327–1331.

Miyata, N., Tsuchida, K., Kaneko, K., Tanaka, M., Otomo, S., 1990. Mechanisms of inhibitory effects of CD-349 and K^+-channel activators on noradnaline-induced contraction and changes in levels of cyclic GMP in rat aorta. Gen. Pharmacol. 21, 665–669.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Moreland, R.B., Goldstein, I., Traish, A., 1998. Sildenafil. A novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. Life Sci. 62, 309–318.

Nishimura, J., Kanaide, H., Nakamura, M., 1987. Characteristics of adrenoceptors and [^{3}H]nitrendipine receptors of porcine vascular smooth muscle: Differences between coronary artery and aorta. Circ. Res. 60, 837–844.

Palmer, R.M.J., Moncada, S., 1989. A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem. Biophys. Res. Commun. 158, 348–352.

Pöch, G., Dittrich, P., Holzmann, S., 1990. Evaluation of combined effects in dose–response studies by statistical comparison with additive and independent interactions. Pharmacol. Methods 24, 311–325.

Pöch, G., Reiffenstein, R.J., Köck, P., Pancheva, S.N., 1995. Uniform characterization of potentiation in simple and complex situations when agents bind to different molecular sites. Can. J. Physiol. Pharmacol. 73, 1574–1581.

Satake, N., Zhou, Q., Morikawa, M., Inoue, M., Shibata, S., 1995. Potentiating effect of nicorandil, an antianginal agent, on relaxation induced by isoproterenol in isolated rat aorta: involvement of cyclic GMP-inhibitable cyclic AMP phosphodiesterase. J. Cardiovasc. Pharmacol. 25, 489–494.

Schultz, J.E., Klumpp, S., Benz, R., Schürhoff-Goeters, M.J.Ch., Schmid, A., 1992. Regulation of adenylyl cyclase from *Paramecium* by an intrinsic potassium conductance. Science 255, 600–603.

Takagi, M., Mochida, H., Noto, T., Yano, K., Inoue, H., Ikeo, T., Kikkawa, K., 2001. Pharmacological profile of T-1032, a novel specific phosphodiestrase type 5 inhibitor, in isolated rat aorta and rabbit corpus cavernosum. Eur. J. Pharmacol. 411, 161–168.

Umans, J.G., Levi, R., 1995. Nitric oxide in the regulation of blood flow and arterial pressure. Annu. Rev. Physiol. 57, 771–790.

Waldman, S.A., Murad, F., 1987. Cyclic GMP synthesis and function. Pharmacol. Rev. 39, 163–196.

Warrington, J.S., Shader, R.I., von Moltke, L.L., Greenblatt, D.J., 2000. In vitro biotransformation of sildenafil (Viagra): identification of human cytochromes and potential drug interactions. Drug Metab. Dispos. 28, 392–397.

Webb, D.J., Freestone, S., Allen, M.J., Muirhead, G.J., 1999. Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist. Am. J. Cardiol. 83 (suppl 5A), 21C–28C.

Webb, D.J., Muirhead, G.J., Wulff, M., Sutton, J.A., Levi, R., Dinsmore, W.W., 2000. Sildenafil citrate potentiates the hypotensive effects of nitric oxide donor drugs in male patients with stable angina. J. Am. Coll. Cardiol. 36, 25–31.

Weir, S.E., Weston, A.H., 1986. The effects of BRL 34915 and nicorandil on electrical and mechanical activity and on ^{86}Rb efflux on rat blood vessels. Br. J. Pharmacol. 88, 121–128.

Zusman, R.M., Morales, A., Glasser, D.B., Osterloh, I.H., 1999. Overall cardiovascular profile of sildenafil citrate. Am. J. Cardiol. 83 (suppl 5A), 35C–44C.