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K_{ATP} channels do not mediate vasodilation by 3-morpholinosydnonimine in goat coronary artery

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Abstract

The present study investigated the role of ATP-sensitive potassium (K_{ATP}) channels in mediating relaxation to the nitric oxide (NO) donor, 3-morpholinosydnonimine (SIN-1) in goat coronary arteries. SIN-1 (10^{-8}–10^{-5} M) caused concentration-dependent relaxations of the coronary artery ring segments contracted with K^+ (30 mM) with an EC_{50} of 6.61×10^{-7} M. Methylene blue (3×10^{-6} M) caused a rightward shift in the concentration–response curve of SIN-1 (10^{-8}–3×10^{-5} M) with a corresponding increase in the EC_{50} (3.62×10^{-6} M) of the nitrovasodilator. While the K_{ATP} channel blocker, glibenclamide (1 and 3×10^{-6} M) caused dose-dependent inhibition of vasorelaxations produced by pinacidil (10^{-8}–10^{-4} M), it had no effect on the vasodilations elicited by SIN-1 (10^{-8}–10^{-5} M) in the coronary arterial smooth muscle. Increasing the extracellular K^+ concentration from 30 mM to 80 mM to reduce the K^+ gradient across the cell membrane, inhibited the relaxations elicited by pinacidil (10^{-8}–10^{-4} M). On the other hand, SIN-1 (10^{-8}–10^{-5} M)-induced relaxations were potentiated in high K^+ (80 mM) compared to those observed at K^+ (30 mM). These results suggest that goat coronary artery vasodilations caused by the NO donor, SIN-1, do not involve K_{ATP} channels. © 1997 Elsevier Science B.V.

Keywords: Coronary artery; SIN-1 (3-morpholinosydnonimine); Nitric oxide (NO); K_{ATP} channel; Vasodilation; Glibenclamide

1. Introduction

Nitric oxide (NO) is believed to account for the biological activity of endothelium-derived relaxing factor (EDRF; Palmer et al., 1987; Ignarro et al., 1987) which plays a vital role in regulating vascular smooth muscle functions. It has been shown that NO stimulates guanylate cyclase (Ignarro et al., 1986) to cause an increase in intracellular cGMP which in turn relaxes vascular smooth muscles through mechanisms that are not completely understood (Lincoln, 1989). Nevertheless, a decrease in intracellular Ca^{2+} involving multiple mechanisms such as stimulation of plasmalemmal Ca^{2+} ATPase, increased sequestration of cytosolic Ca^{2+} through activation of Ca^{2+} ATPase pump, attenuation of voltage-dependent calcium channels, has been implicated as the primary mechanism in cGMP-mediated vasodilation due to NO (Griffith, 1994). Membrane hyperpolarization is also considered as a mechanism in NO relaxations (Tare et al., 1990).

In view of the critical role of K^+ channels in regulating membrane potential and tone of arterial smooth muscles (Nelson and Quayle, 1995), in recent years attention has been focused on ascertaining the role of different K^+ channels in NO-induced relaxation of vascular smooth muscles (Khan et al., 1993; Bolotina et al., 1994). However, the involvement of various types of K^+ channels in mediating relaxation/hyperpolarization in response to NO is controversial. For example, a role for K_{Ca} channels in vasorelaxation caused by NO has been shown in rabbit superior mesenteric arteries (Khan et al., 1993) and aorta (Bolotina et al., 1994). On the contrary, Murphy and Brayden (1995) recently showed that K_{ATP} channels, but not K_{Ca} channels, were involved in NO-evoked hyperpolarizations of the smooth muscle cells of rabbit mesenteric arteries. Consistent with these results, the K_{ATP} channel blocker glibenclamide was shown to abolish hyperpolarization in response to NO in guinea-pig coronary artery. Interestingly, however, it did not affect the amplitude of NO relaxations in the same tissue (Parkington et al., 1995). Further, patch-clamp studies provide evidence that K_{ATP} channels are activated by NO in porcine coronary arterial smooth muscle cells (Miyoshi et al., 1994). Notwithstanding such discrepancies in NO action on different K^+

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channels, Nelson and Quayle (1995) proposed a hypothesis which suggests that NO is acting in part through the activation of K_{ATP} channels to mediate vasodilation in endotoxin shock. In view of its sufficient clinical relevance, this hypothesis needs to be examined using blood vessels taken from different animal species.

The present investigation was undertaken to determine whether K_{ATP} channels are involved in the relaxant effect of NO in vascular smooth muscle of isolated goat coronary artery. 3-Morpholinosydnonimine (SIN-1), which has been shown to relax vascular smooth muscles through the release of NO and elevation of cGMP (Feelisch et al., 1989; Moncada and Higgs, 1995), was used as a prototype NO donor in the present study. This organic nitrovasodilator was used in a recent study (Murphy and Brayden, 1995) which showed that NO released from SIN-1 hyperpolarized vascular smooth muscles in rabbit mesenteric arteries by activating K_{ATP} channels with the accumulation of cGMP as an intermediate step, but no role for K_{ATP} channel involvement in relaxation due to SIN-1 has been demonstrated. In order to examine the role of K_{ATP} channels in the current investigation, the K_{ATP} channel opener pinacidil (Hamilton and Weston, 1989) and K_{ATP} channel blocker glibenclamide (Standen et al., 1989) were used. Several investigators have used goats as an ideal animal model for coronary circulation studies (Dankelman et al., 1989a,b, 1994; Toda et al., 1996). Further, like in other species of animals (Eckman et al., 1992; Imamura et al., 1992), K_{ATP} channels have been shown to play an important role in regulating coronary blood flow in goats (Dankelman et al., 1994). Therefore, we employed goat coronary artery as a model of vascular smooth muscle in the present study.

2. Materials and methods

2.1. Tissue preparation

Goat hearts were obtained from a local abattoir immediately (15 min) after slaughter and were transported to the laboratory in oxygenated cold physiological saline solution (PSS) of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 11.9; KH_2PO_4, 1.2; glucose, 11.1. The circumflex and the anterior descending branch of the left coronary artery were isolated from each heart. The arteries were cleaned of fat and connective tissue and cut into rings of about 3 mm length and 1.5 mm outer diameter for tension experiments.

2.2. Tension experiments

Coronary artery rings were mounted between two stainless-steel angular hooks and suspended in an organ bath containing 20 ml of PSS, continuously bubbled with air (pH 7.4) at 37 ± 0.5°C. The tissues were equilibrated under a resting tension of 1.5 g for 90 min during which the PSS was changed at every 15 min. Isometric contractions were recorded by a force transducer connected to an ink-writing oscillograph (Recorders and Medicare, India).

2.3. Experimental protocol

2.3.1. Concentration–response curves of SIN-1

After the equilibration period, coronary artery rings were contracted with 30 mM K^+ saline solution of the following composition (mM): NaCl, 92.7; KCl, 30.0; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 11.9; NaH_2PO_4, 1.2; glucose, 11.1. This concentration of K^+ has been used in previous studies (Hamilton and Weston, 1989; Makijuna et al., 1994) to elicit relaxations with potassium channel openers in vascular smooth muscles. At the plateau of K^+ contraction, SIN-1 was added to the organ bath cumulatively at an increment of 0.5 log unit to generate concentration-dependent relaxations of the arterial ring segments. Parallel control tissues were used without SIN-1 to examine the effect of time and vehicle on the plateau phase of K^+ contraction.

2.3.2. Effect of methylene blue on SIN-1-induced relaxations

Methylene blue is known to destroy the biological activity of NO (Wolin et al., 1990) or inhibit soluble guanylate cyclase, an enzyme activated by NO (Ignarro et al., 1986). Therefore, to investigate the contribution of NO to the coronary artery vascular smooth muscle relaxation by SIN-1, methylene blue was used. After a control concentration–response curve to SIN-1 was generated in K^+ (30 mM)-precontracted ring segments, the tissues were washed with PSS several times and equilibrated with 3 μM methylene blue for 30 min and rechallenged with K^+ (30 mM). At the plateau of the second K^+ contraction, SIN-1 was added cumulatively as described above.

2.3.3. Effect of glibenclamide on SIN-1, sodium nitroprusside and pinacidil relaxations

These experiments were designed to assess the functional role of vascular K_{ATP} channels in mediating the coronary artery smooth muscle relaxations caused by SIN-1 and sodium nitroprusside. A cumulative concentration–response curve for each nitrovasodilator was generated by adding the drug to the bath at an increment of 0.5 log unit in the absence or presence of glibenclamide (1 and 3 μM). The antagonism of the relaxant responses of SIN-1 and sodium nitroprusside by glibenclamide was studied using the following protocol. After the equilibration period of 90 min, either control concentration–response curves to the vasodilators were elicited or the tissues were exposed to different concentrations of glibenclamide before the responses to SIN-1 or sodium nitroprusside were evoked in K^+ (30 mM)-precontracted coronary arteries.

For comparison, cumulative concentration-dependent

relaxations with pinacidil, a known activator of K_{ATP} channels (Hamilton and Weston, 1989), were elicited in the presence and absence of glibenclamide (0.1, 1 and 3 μM), a potent blocker of K_{ATP} channels (Standen et al., 1989) on K^+ (30 mM)-contracted tissues.

2.3.4. Effect of tetraethylammonium on SIN-1 responses

To see the possibility of the involvement of other potassium channels such as K_{Ca} channels in the vasodilation caused by SIN-1 in goat coronary artery, tetraethylammonium, a moderately specific inhibitor of K_{Ca} channels (Beech and Bolton, 1989; Brayden and Nelson, 1992), was used. In this series of experiments, after the control concentration–response curve to SIN-1 was obtained in K^+ (30 mM)-contracted ring segments, the tissues were washed several times and equilibrated with tetraethylammonium (1 mM) for 30 min before the second concentration–response curve to SIN-1 was elicited.

2.3.5. Effect of high K^+ (80 mM) saline solution on responses to SIN-1 and pinacidil

High K^+ (80 mM), that attenuates the K^+ gradient across the cell membrane, is one of the experimental protocols to assess the involvement of K^+ channels in the mechanism of action of a vasodilator (Meisheri et al., 1990). In the present set of experiments, coronary artery ring segments were contracted with K^+ (80 mM) solution (prepared by equimolar replacement of Na^+ in the PSS) and at the plateau of the contraction, either SIN-1 or pinacidil was added cumulatively at an increment of 0.5 log units to generate concentration-dependent relaxations. The tissues were pretreated with phenoxybenzamine (1 μM) to prevent any effect of endogenously released noradrenaline. Some experiments were done in the absence of phenoxybenzamine in order to determine the per se effect of phenoxybenzamine in altering the sensitivity of the vasodilators in high K^+ (80 mM) solution.

2.4. Drugs

Glibenclamide was a gift from Hoechst, Germany and was prepared as a stock solution of 10^{-2} M in dimethyl sulphoxide (DMSO). Methylene blue (Sigma) was dissolved in double glass distilled water to get a stock solution of 10^{-2} M. Pinacidil was a gift from Leo Pharmaceutical Products, Denmark and was prepared as a stock solution of 10^{-2} M in 0.1 M HCl. 3-Morpholinosydnonimine (SIN-1) was a generous gift from Hoechst, Germany and was prepared as a stock solution of 10^{-2} M in PSS afresh every day. Sodium nitroprusside (Merck, India) was prepared as a stock solution of 10^{-2} M in distilled water every day. The stock solutions of the nitrovasodilators were stored at 4°C and were protected from light. Tetraethylammonium (Merck, Germany) was dissolved in distilled water to get a stock solution of 10^{-1} M.

2.5. Statistics

The results (absolute force/per cent relaxations) are presented as means ± S.E. Student's *t*-test was used to determine the level of significance. The EC_{50} values of the vasodilators were calculated by regression analysis and were expressed as geometric mean with their 95% confidence limits (Snedecor and Cochran, 1967).

3. Results

3.1. Relaxations caused by SIN-1

The concentration–response curves for SIN-1 are shown in Fig. 1. SIN-1 (10^{-8}–10^{-5} M), added cumulatively at an increment of 0.5 log unit caused concentration-dependent relaxations of goat coronary artery rings contracted with K^+ (30 mM) PSS (absolute force, 0.94 ± 0.05 g; $n = 14$)

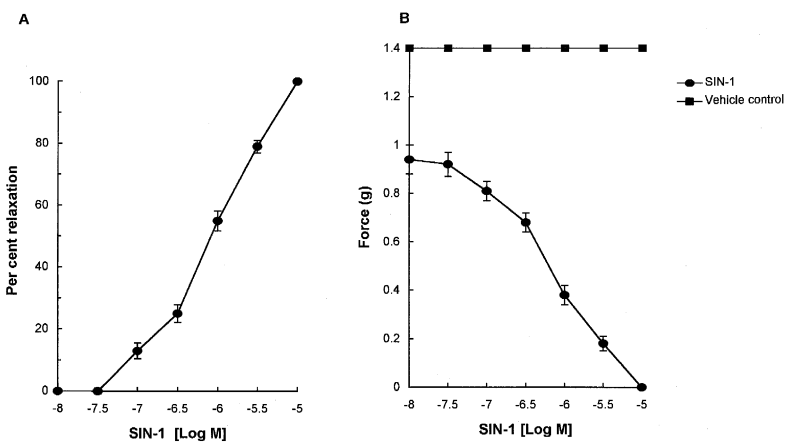

Fig. 1. (A) Relaxations to SIN-1 (10^{-8}–10^{-5} M) of goat coronary artery ring segments contracted with K^+ (30 mM) saline solution ($n = 14$). (B) The reversal of K^+ (30 mM) contraction (expressed as force in g) in a dose-dependent manner by SIN-1 (10^{-8}–10^{-5} M; $n = 14$) and the effect of an identical volume of the vehicle (PSS), added cumulatively, on the plateau phase of K^+ (30 mM) contraction ($n = 2$).

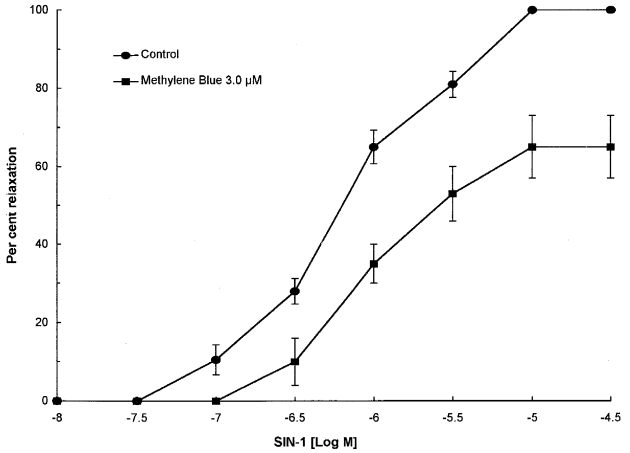

Fig. 2. Effect of methylene blue (3×10^{-6} M) on the concentration-dependent relaxations of SIN-1 (10^{-8}–3×10^{-5} M) on coronary arteries contracted with K^+ (30 mM). The vertical bars represent the standard error of the mean ($n=4$ for each group).

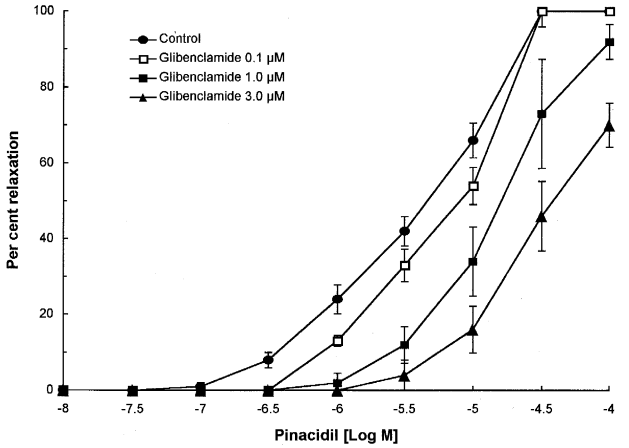

Fig. 3. Effect of glibenclamide (0.1, 1 and 3×10^{-6} M) on the concentration–response curves of pinacidil (10^{-8}–10^{-4} M) in coronary arterial segments contracted with K^+ (30 mM). The vertical bars represent the standard error of the mean ($n=4$–6 for each group).

with the maximum relaxation (100%) occurring at 10^{-5} M (Fig. 1A). The EC_{50} for SIN-1 was 6.61×10^{-7} M (95% C.L., 5.49–7.94×10^{-7} M; $n=14$). Fig. 1B shows the dose-dependent reversal of K^+ contracture (expressed as force in g) by SIN-1 and the time-matched plateau phase of K^+ (30 mM) contraction to which an identical volume of the vehicle (PSS) was added instead of the drug.

3.2. Effects of methylene blue on the responses of SIN-1

In the next series of experiments the role of NO in mediating the relaxations of K^+ (30 mM)-contracted coronary arterial rings by SIN-1 was examined. The results are presented in Fig. 2. Equilibration of the tissues for 30 min with methylene blue (3×10^{-6} M) potentiated the K^+ (30 mM) contractions by 25% and caused a rightward shift in the concentration–response curves of SIN-1 with a corresponding increase in the EC_{50} of the nitrovasodilator. Thus, the EC_{50} values for SIN-1 were 6.61×10^{-7} M (95% C.L., 4.47–9.77×10^{-7} M; $n=4$) and 3.39×10^{-6} M (95% C.L., 1.12×10^{-6}–1.23×10^{-5} M; $n=4$) in the absence and presence of methylene blue, respectively. Unlike the controls, wherein SIN-1 (10^{-5} M) caused 100% relaxation of K^+ (30 mM)-contracted tissues, methylene blue at the concentration used, reduced the relaxant response of the vasodilator by 35%. Increasing the concentration of SIN-1 to 3×10^{-5} M did not further relax the tissues.

3.3. Effects of glibenclamide on relaxations induced by pinacidil, SIN-1 and sodium nitroprusside

The dose-dependent effect of glibenclamide on concentration–response curves of pinacidil are shown in Fig. 3. Pinacidil (1×10^{-8}–3×10^{-5} M), a known vascular K_{ATP}

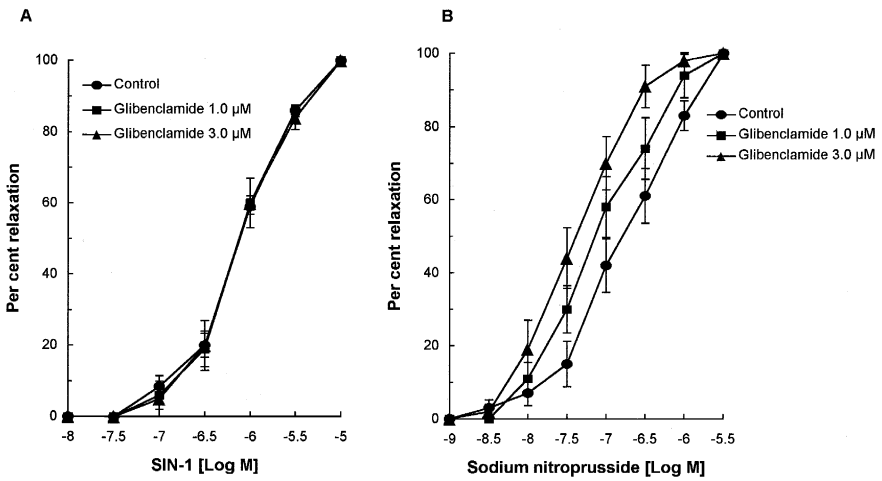

Fig. 4. (A) Effect of glibenclamide (1 and 3×10^{-6} M) on concentration-dependent relaxation elicited by SIN-1 (10^{-8}–10^{-5} M) on coronary arteries contracted with K^+ (30 mM; $n=4$ for each group). (B) The effects of glibenclamide (1 and 3×10^{-6} M) on concentration-dependent relaxations to sodium nitroprusside (10^{-9}–3×10^{-6} M) in K^+ (30 mM)-contracted coronary arterial segments ($n=6$ for each group). The vertical bars represent the standard error of the mean.

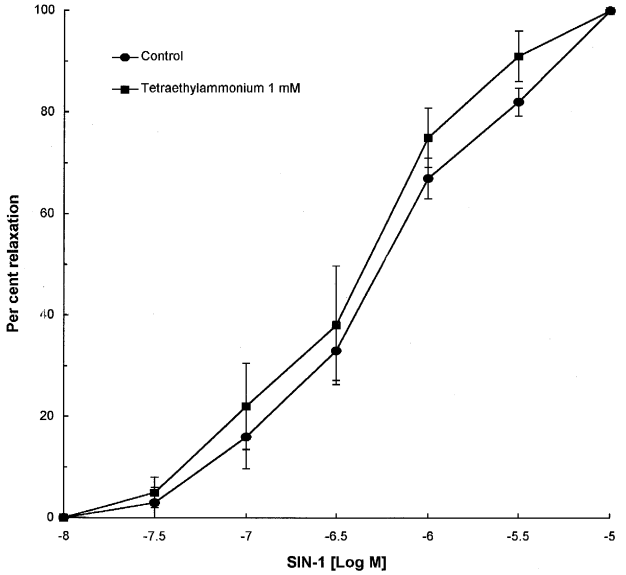

Fig. 5. Effect of tetraethylammonium (1 mM) on the concentration-dependent relaxations produced by SIN-1 (10^{-8}–10^{-5} M) in coronary artery rings contracted with K^+ (30 mM). The vertical bars represent the standard error of the mean ($n = 4$–5 for each group).

channel opener, produced concentration-dependent relaxations of the coronary artery rings contracted with K^+ (30 mM) saline solution, with the maximum relaxation achieved at 3×10^{-5} M. Pretreatment of the tissues with glibenclamide (0.1, 1, and 3×10^{-6} M) for 30 min caused a dose-dependent rightward parallel shift in the concentration–response curves of pinacidil. Thus, the EC_{50} values for pinacidil were 2.69×10^{-6} M (95% C.L., $1.48–4.9 \times 10^{-6}$ M; $n = 6$), 4.95×10^{-6} M (95% C.L., $3.8–6.61 \times 10^{-6}$ M; $n = 4$), 2.15×10^{-5} M (95% C.L., $1.02–4.47 \times 10^{-5}$ M; $n = 4$) and 3.21×10^{-5} M (95% C.L., 1.17–8.91 $\times 10^{-5}$ M; $n = 5$) in the control and in the presence of 0.1, 1, and 3×10^{-6} M of glibenclamide, respectively.

As can be seen from Fig. 4A, glibenclamide (1 and 3×10^{-6} M), which markedly inhibited the responses to pinacidil, had no effect on the concentration-dependent relaxations evoked by SIN-1 (1×10^{-8}–1×10^{-5} M) in K^+ (30 mM)-contracted tissues. The EC_{50} value for SIN-1 was 6.61×10^{-7} M (95% C.L., $5.49–7.94 \times 10^{-7}$ M; $n = 4$) in control compared to 7.64×10^{-7} M (95% C.L., $5.25–10.96 \times 10^{-7}$ M; $n = 4$) and 7.94×10^{-7} M (95% C.L., $3.98–15.84 \times 10^{-7}$ M; $n = 4$) in the presence of 1 and 3×10^{-6} M of glibenclamide, respectively. Glibenclamide (1 and 3×10^{-6} M) had no effect on the course of relaxation induced by SIN-1 (3×10^{-6} M; data not shown) in arterial rings contracted with K^+ (30 mM).

Sodium nitroprusside (10^{-9}–3×10^{-6} M), another nitric oxide donor, relaxed the K^+ (30 mM)-precontracted coronary artery segments in a concentration-related manner (Fig. 4B). Complete relaxation with the nitrovasodilator was achieved at 3×10^{-5} M. Pretreatment of the tissues with glibenclamide (1 and 3×10^{-6} M) caused a dose-dependent leftward shift of the concentration–response curves of sodium nitroprusside. Hence, the EC_{50} of the vasodilator was 1.48×10^{-7} M (95% C.L., 0.66–3.33 $\times 10^{-7}$ M; $n = 6$) in control tissues compared to 7.94×10^{-8} M (95% C.L., 2.63×10^{-8} M–2.3×10^{-7} M; $n = 4$) and 4.26×10^{-8} M (95% C.L., $1.86–9.77 \times 10^{-8}$ M; $n = 6$) in the presence of glibenclamide 1 and 3×10^{-6} M, respectively.

3.4. Effects of tetraethylammonium on the responses of SIN-1

Fig. 5 depicts the results of the sensitivity of SIN-1-induced relaxations to tetraethylammonium (1 mM). Pre-

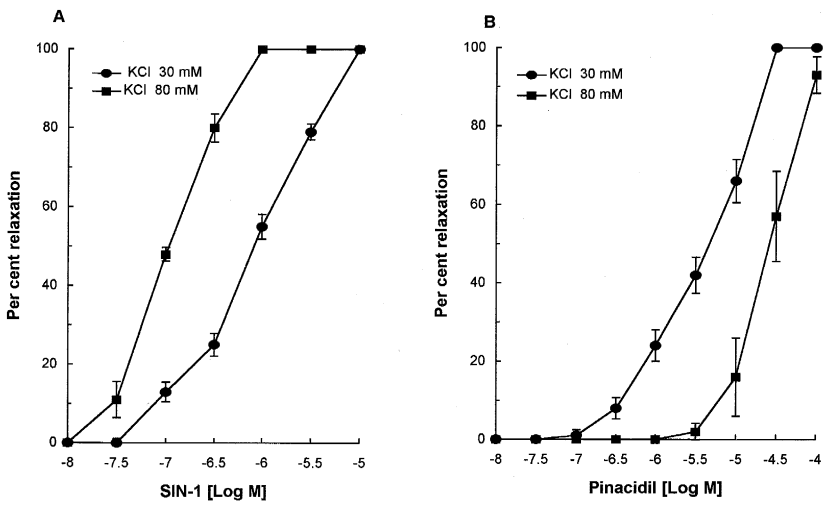

Fig. 6. (A) Concentration-dependent relaxations to SIN-1 (10^{-8}–10^{-5} M) of coronary artery rings contracted with high K^+ (80 mM) saline solution ($n = 4$). The data that show the responses of the tissue to SIN-1 in K^+ (30 mM) solution were taken from Fig. 1A. (B) The concentration-dependent relaxations elicited by pinacidil (10^{-8}–10^{-4} M) in coronary arterial rings contracted either with high K^+ (80 mM; $n = 4$) saline solution or with K^+ (30 mM; data taken from Fig. 3). The vertical bars represent the standard error of the mean. Note that the contractions with high K^+ (80 mM) saline solutions were elicited in the presence of phenoxybenzamine (10^{-6} M).

treatment of the tissues with tetraethylammonium (1 mM) for 30 min had no significant effect on the concentration-dependent relaxations produced by SIN-1 (10^{-8}–10^{-5} M) in the coronary artery rings contracted with K^+ (30 mM). Thus, the EC_{50} of SIN-1 in control was 6.76×10^{-7} M (95% C.L., 6.46–7.08×10^{-7} M; $n = 5$), which was not significantly different from that obtained in the presence of TEA (4.03×10^{-7} M; 95% C.L., 1.3–12.4×10^{-7} M; $n = 4$).

3.5. Effects of high K^+ (80 mM) PSS on the relaxations of SIN-1 and pinacidil

Fig. 6 illustrates the comparative effects of K^+ (30 mM) and K^+ (80 mM) solutions on relaxations elicited by SIN-1 and pinacidil in goat coronary artery. As shown in Fig. 6A, a leftward shift in the concentration–response curve to SIN-1 (1×10^{-8}–1×10^{-5} M) was observed in tissues contracted with K^+, 80 mM (absolute force, 1.60 ± 0.19 g; $n = 6$) compared to those exposed to K^+ (30 mM). Hence, the EC_{50} values of SIN-1 to cause vasodilation were 1.12×10^{-7} M (95% C.L., 0.98–1.29×10^{-7} M; $n = 4$) and 6.61×10^{-7} M (95% C.L., 5.49–7.94×10^{-7} M; $n = 14$) in K^+ (80 mM) and K^+ (30 mM) PSS, respectively. On the contrary, the concentration–response curves obtained with pinacidil (1×10^{-8}–1×10^{-4} M) in coronary artery ring segments contracted by K^+ (30 mM) PSS were shifted to right in K^+ (80 mM) PSS, with a corresponding increase in its EC_{50}. Thus, the EC_{50} of pinacidil was 2.69×10^{-6} M (95% C.L., 1.48–4.9×10^{-6} M; $n = 6$) in K^+ (30 mM), which was significantly less than that obtained in K^+ (80 mM) PSS (2.75×10^{-5} M; 95% C.L., 2.59–2.91×10^{-5} M; $n = 4$). In some experiments, relaxations to SIN-1 (10^{-8}–10^{-5} M) and pinacidil (10^{-8}–10^{-4} M) were elicited on K^+ (80 mM)-contracted coronary arteries in the absence of phenoxybenzamine. The EC_{50} values for SIN-1 and pinacidil were 1.09×10^{-7} M and 1.18×10^{-5} M, respectively, which were not significantly different from those obtained in the presence of the α-adrenoceptor blocker.

4. Discussion

The present study provides a comprehensive analysis of the role of K_{ATP} channel and other K^+ channels in mediating relaxations by SIN-1 (and by implication of NO released from the organic nitrovasodilator) in goat coronary artery smooth muscle. The main findings are: (1) The relaxations evoked by SIN-1 were antagonised by methylene blue, an inactivator of the biological activity of NO, while (2) glibenclamide, at the concentrations that caused dose-dependent inhibition of vasorelaxations produced by the K_{ATP} channel opener pinacidil failed to attenuate relaxations due to SIN-1. (3) Tetraethylammonium at the concentration known to block K_{Ca} channels (Brayden and Nelson, 1992) did not antagonise the relaxant responses to SIN-1. (4) In comparison to relaxations elicited by pinacidil in tissues contracted with K^+ (30 mM), vasodilations achieved by the potassium channel opener were significantly reduced in arterial rings contracted with high K^+ (80 mM), which is known to abolish the K^+ gradient across the cell membrane. On the other hand, SIN-1-induced relaxations were potentiated in K^+ (80 mM) solution compared to those obtained with K^+ (30 mM). The evidence in support of each observation is discussed below.

The inhibition by methylene blue of SIN-1-induced relaxations in the goat coronary artery suggests that the pharmacological effects of the nitric oxide donor are mediated by NO. The involvement of an NO mechanism is supported by the observations of an earlier study which showed that the vasorelaxation caused by SIN-1 resulted from the generation of NO in vascular smooth muscles (Feelisch et al., 1989). In a recent study, Murphy and Brayden (1995) also reported that the responses of the vascular smooth muscle to SIN-1 were mediated by NO.

It is generally believed that hyperpolarization that occurs as a result of K^+ channel opening can subsequently cause voltage-dependent Ca^{2+} channels to close resulting in vasodilation (Nelson et al., 1988). Recently, the K_{ATP} channel blocker glibenclamide has been shown to abolish hyperpolarization of vascular smooth muscles to SIN-1 (Murphy and Brayden, 1995). However, no information is available to demonstrate the involvement of K_{ATP} channels in relaxations of vascular smooth muscles by the nitrovasodilator. This served as the basis to examine the hypothesis that K_{ATP} channels are involved in SIN-1 (by implication of NO)-induced relaxations of vascular smooth muscles. To ascertain the functionality of K_{ATP} channels in goat coronary artery preparations, pharmacologically relevant concentrations of the K_{ATP} channel ligands pinacidil and glibenclamide were used. SIN-1 (1×10^{-8}–1×10^{-5} M) and pinacidil (1×10^{-8}–3×10^{-5} M) relaxed goat coronary artery segments contracted with K^+ (30 mM) in a concentration-dependent manner. The relaxations due to SIN-1 were not blocked by 1 and 3 μM of glibenclamide. On the other hand, glibenclamide 1 and 3 μM caused a dose-dependent parallel shift to right of the concentration–response curves of pinacidil. While these results suggest the presence of functional K_{ATP} channels in the goat coronary artery and confirm an earlier observation in this species (Dankelman et al., 1994), the finding that glibenclamide failed to attenuate the relaxations of SIN-1 leads us to suggest that K_{ATP} channel activation is unlikely to contribute to vasorelaxations caused by the nitrovasodilator in goat coronary artery. In order to further substantiate this observation, glibenclamide sensitivity of the relaxations due to another nitrovasodilator, sodium nitroprusside, was studied. Surprisingly, glibenclamide at 1 and 3 μM potentiated the responses to sodium nitroprusside (10^{-8}–3×10^{-6} M) resulting in a leftward shift in the

concentration–response curves of the vasodilator in goat coronary artery segments. While the current investigation cannot explain the mechanisms of potentiation, it, however, rules out the possibility of any role for K_{ATP} channels in mediating relaxations to NO released from these nitrovasodilators. Thus, the results of the present study are supported by the findings of the previous studies wherein glibenclamide has been shown to have no inhibitory effect on NO- and nitroglycerin-induced relaxations in rabbit superior mesenteric arteries (Khan et al., 1993) and vasorelaxation produced by NO in guinea-pig coronary artery (Parkington et al., 1995). On the contrary, the findings of the present study are in contrast to the hypothesis proposed by Nelson and Quayle (1995) implicating K_{ATP} channels in mediating vasorelaxations due to NO.

It is evident that there is little consensus on the involvement of K_{Ca} channels in the pharmacological actions of NO in vascular smooth muscles. Cabell et al. (1994) showed that large conductance K_{Ca} channels were not involved in cGMP-mediated relaxations in canine coronary arteries, since relaxations due to endothelium-dependent (bradykinin and acetylcholine) and endothelium-independent (nitroprusside) stimulation of cGMP were not affected by tetraethylammonium (1 mM), that preferentially blocks K_{Ca} channels (Brayden and Nelson, 1992). On the contrary, K_{Ca} channels were shown to be involved in relaxations due to NO and nitroglycerin in rabbit superior mesenteric arteries (Khan et al., 1993) and rabbit aorta (Bolotina et al., 1994). In the present study, however, the fact that tetraethylammonium (1 mM) failed to attenuate the relaxations by SIN-1 suggests that K_{Ca} channel modulation is not an important component in NO-mediated vasodilation in goat coronary artery.

If the relaxations due to SIN-1 are likely to result from an increase in K^+ conductance, they could be inhibited when the K^+ gradient across the cell membrane is reduced. To test this possibility, concentration–response curves to SIN-1 and pinacidil were elicited in tissues contracted with high K^+ (80 mM). As expected, the relaxations induced by pinacidil were markedly inhibited in K^+ (80 mM) compared to those observed in tissues contracted with K^+ (30 mM), which is consistent with the inhibitory effect of increasing concentrations of external K^+ on the relaxation caused by potassium channel openers (Meisheri et al., 1990).

However, the observation that pinacidil at higher concentrations continued to relax the goat coronary artery vascular smooth muscle in high K^+ (80 mM) indicates that a component of relaxation in response to the potassium channel opener involved mechanisms other than an effect on K^+ conductance as reported in other vascular smooth muscles (Erne and Hermsmeyer, 1991). Contrary to the observations made with pinacidil, relaxations evoked by SIN-1 were potentiated in coronary artery segments contracted with K^+ (80 mM) compared to those stimulated with K^+ (30 mM). Although the experiments done in the present investigation cannot explain the mechanisms of potentiation, these findings indicate that the relaxations of SIN-1 are not due to an increase in K^+ conductance by the vasodilator in the goat coronary artery vascular smooth muscle.

It is, however, possible that SIN-1 antagonized voltage-dependent Ca^{2+} channels (opened during high K^+ depolarization) to relax the coronary artery smooth muscle. Recent evidence shows that SIN-1 inhibits mammalian cardiac calcium current through cGMP-dependent protein kinase (Wahler and Dollinger, 1995). However, the participation of other possible mechanisms like lowering of $[Ca^{2+}]_i$, as a result of reduced $[Ca^{2+}]_i$ release or enhanced sequestration/efflux of $[Ca^{2+}]_i$, by SIN-1 cannot be ruled out, as suggested for other nitrovasodilators (Griffith, 1994). The NO donor sodium nitroprusside has also been shown to inhibit Ca^{2+} current and Ca^{2+} influx in several vascular smooth muscles (Magliola and Jones, 1990; Clapp and Gurney, 1991).

The fact that glibenclamide, tetraethylammonium and increased extracellular K^+ (80 mM) were ineffective in antagonizing the relaxations produced by SIN-1 implicates that the relaxations caused by the nitrovasodilator through NO release may not involve an increase in K^+ conductance in goat coronary artery smooth muscle. Our results are supported by previous studies wherein NO-induced relaxations in guinea-pig coronary artery were shown to be resistant to glibenclamide and tetraethylammonium (Parkington et al., 1995). Similarly, in other blood vessels (rabbit abdominal aorta and carotid arteries) too, endogenous NO released by acetylcholine was shown to induce complete relaxation in the presence of glibenclamide and charybdotoxin (Cowan et al., 1993).

In conclusion, the findings of the present study suggest that the goat coronary artery vasodilations due to SIN-1 (possibly mediated by NO) are independent of K_{ATP} channel activation. Despite the fact that both NO and K_{ATP} channels have been shown to play significant roles in conditions like hypoxic vasodilation and hypotension in endotoxin shock (Landry and Oliver, 1992), the findings of the present study rule out any possible role for K_{ATP} channels in mediating vasorelaxation to NO.

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