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Hydroxylamine-induced relaxation inhibited by K^+ channel blockers in rat aortic rings

Yu Huang *

Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China

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Abstract

Hydroxylamine, a putative endogenous nitric oxide donor, relaxed rat aorta in a concentration-dependent manner (0.01–30 μM). Removal of endothelium or pretreatment of aortic tissue with N^G-nitro-L-arginine (L-NOARG, 100 μM) did not affect the relaxant effect of hydroxylamine but L-NOARG at 100 μM abolished the acetylcholine-induced relaxation. Methylene blue (10 μM) significantly reduced the relaxant effect of hydroxylamine in endothelium-denuded arteries. Tetrapentylammonium ions (0.3–3 μM), tetraethylammonium ions (1–3 mM) and charybdotoxin (100 nM) reduced the relaxant effect of hydroxylamine in the endothelium-denuded arteries while glibenclamide (3 μM) had no effect. Neither tetrapentylammonium nor tetraethylammonium ions affected relaxations induced by forskolin and verapamil. The effects of tetrapentylammonium ions (3 μM) and charybdotoxin (100 nM) were additive. Tetrapentylammonium ions (3 μM), tetraethylammonium ions (3 mM) and charybdotoxin (100 nM) decreased the relaxation induced by sodium nitroprusside in the endothelium-denuded arteries while glibenclamide (3 μM) had no effect. The concentration–relaxation curve for the relaxant effect of hydroxylamine was shifted to the right in the presence of high extracellular K^+ (15–60 mM). Neither tetrapentylammonium ions (3 μM) nor charybdotoxin (100 nM) affected hydroxylamine-induced relaxation of the endothelium-denuded aorta precontracted with 60 mM K^+. These results indicate that hydroxylamine relaxes the rat aorta partially through activation of tetrapentylammonium-, tetraethylammonium- and charybdotoxin-sensitive K^+ channels and its action is comparable with that of sodium nitroprusside, an exogenous nitric oxide donor. The endothelium is not involved in the aortic response to hydroxylamine.

Keywords: Hydroxylamine; Sodium nitroprusside; Tetrapentylammonium; Charybdotoxin; Glibenclamide; K^+ channel; Nitric oxide (NO); Relaxation; Aorta; (Rat)

1. Introduction

The movement of K^+ ions across the plasma membrane of arterial smooth muscle is an important determinant of the membrane potential, which in turn regulates the influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels and thus muscle contractility. Activation of K^+ channels causes membrane hyperpolarization, which closes voltage-sensitive Ca^{2+} channels and relaxes vascular smooth muscle (Nelson et al., 1990b). Both Ca^{2+}-activated K^+ (K_{Ca}) channels and ATP-sensitive K^+ (K_{ATP}) channels with distinct pharmacological properties have been extensively studied in arterial smooth muscle (Brayden and Nelson, 1992; Langton et al., 1991; Nelson et al., 1990b; Standen et al., 1989).

In response to hormonal stimuli which trigger Ca^{2+} influx (Himmel et al., 1993), the endothelial cells in intact arteries regulate the contractility of the underlying arterial smooth muscle by releasing vasoactive factors, which cause relaxation via a variety of mechanisms. Nitric oxide, formed enzymatically from the terminal guanidine nitrogen atoms of L-arginine in the endothelium (Moncada et al., 1991), is the best known endothelium-derived relaxing factor. Nitric oxide, apart from stimulating guanylate cyclase in smooth muscle, may modulate the activity of ionic channels on the plasma membrane. For example, nitric oxide hyperpolarizes rabbit mesenteric arteries through activation of K_{ATP} channels (Murphy and Brayden, 1995) and activates K_{Ca} channels indirectly by increasing the activity of cyclic GMP-dependent protein kinase in cerebral and coronary arteries (Archer et al., 1994; Robertson

* Corresponding author. Fax: +86-852-2603-5022; e-mail: yu-huang@cuhk.edu.hk

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et al., 1993; Taniguchi et al., 1993). Hydroxylamine is suggested to be an intermediate product of the pathway for the oxidative conversion of L-arginine to nitric oxide and thus may be an endogenous nitric oxide donor (DeMaster et al., 1989; Thomas and Ramwell, 1989). This mechanism may account for its relaxant effect on rabbit and rat aorta (Kruszyna et al., 1982; Rapoport and Murad, 1984). Hydroxylamine requires a catalase-dependent reaction to give rise to nitric oxide (Craven et al., 1979; DeMaster et al., 1989), whilst sodium nitroprusside releases nitric oxide through a non-enzymatic process. More recently, hydroxylamine was shown to activate K_{ATP} channels in pancreatic β-cells (Antoine et al., 1996). However, the exact mechanisms underlying the relaxant effect of hydroxylamine are still unclear.

The present investigation was intended to determine whether activation of K_{ATP} or K_{Ca} channels contributes toward the vasorelaxant responses induced by hydroxylamine and the exogenous nitric oxide donor sodium nitroprusside in rat isolated aortic rings. Specifically, the relaxant responses to each nitric oxide donor were compared in the absence and presence of the blockers of K_{ATP} and K_{Ca} channels.

2. Materials and methods

2.1. Tissue preparation

Male Sprague–Dawley rats ($\sim$250 g) were killed by cervical dislocation and bled. The thoracic aorta was dissected out and surrounding connective tissues were carefully removed. Four aortic rings $\sim$3 mm in length were prepared from each rat and placed in 10-ml organ baths containing Krebs–Henseleit solution (mM): NaCl 119, KCl 4.7, $CaCl_2$ 5, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.2, D-glucose 11.1, ascorbic acid 0.2. The bath solution was constantly gassed with a mixture of 95% O_2 and 5% CO_2, and maintained at 37°C. The aortic rings were allowed to equilibrate under 1.5 g resting tension for 90 min before experiments were carried out, during which time muscle tension was readjusted to 1.5 g. Contraction was recorded isometrically by means of Grass FT03 force transducers (Grass Instrument) and stored on 486 Macintosh computer using Maclab software (version 3). In most experiments, the endothelium was removed mechanically by rubbing the lumen of the artery with plastic tubing. The removal of the endothelium was deemed successful if no relaxation was induced in response to acetylcholine (1 μM) at the start of each experiment. In endothelium-intact arteries 1 μM acetylcholine induced approximately 70% relaxation.

2.2. Experimental protocols

Thirty minutes after being set up in organ baths, the preparations were first contracted with a single concentration of phenylephrine (0.1 μM) to test their contractility, after which time they were washed several times with Krebs–Henseleit solution to restore tension to the precontracted level. In the first set of experiments, tissues containing an intact endothelium were incubated for 30 min with N^G-nitro-L-arginine (L-NOARG, 100 μM) and then the concentration-dependent effect of hydroxylamine was examined on the phenylephrine-contracted artery. In a second group of experiments, the steady contractions of aortic rings in the absence of endothelium were induced by phenylephrine (0.1 μM), and hydroxylamine or sodium nitroprusside was added cumulatively to obtain concentration–relaxation curves under control conditions and in the presence of methylene blue (10 μM) or various putative K^+ channel blockers (1–3 mM TEA^+, 0.3–3 μM TPA^+, 100 nM charybdotoxin, 3 μM glibenclamide, 30 min contact time). The effects of putative K^+ channel blockers were also tested on relaxations induced by forskolin and verapamil in endothelium-denuded preparations. In the last series of experiments, a similar magnitude of sustained tension was induced in the presence of 5.9, 15, 30 and 60 mM extracellular K^+ by lowering the concentration of phenylephrine (from 0.1 to 0 μM). In experiments with the high K^+ solution, Na^+ was replaced by an equimolar concentration of K^+ to keep a constant ion strength. Each set of experiments was performed with arteries from different rats.

2.3. Chemicals

The following compounds were used in the present study: phenylephrine hydrochloride, hydroxylamine hydrochloride, forskolin (Research Biochemicals, Natick, MA, USA), tetrapentylammonium ions chloride, tetraethylammonium ions chloride, charybdotoxin, glibenclamide, sodium nitroprusside, N^G-nitro-L-arginine, methylene blue, ($\pm$)-pinacidil, verapamil and acetylcholine chloride (Sigma, St. Louis, MO, USA). All drugs were dissolved in Krebs solution except for glibenclamide, pinacidil, forskolin and verapamil, which were dissolved in dimethyl sulfoxide. Dimethyl sulfoxide at a final concentration of 0.2% (v/v) did not affect the phenylephrine-induced contraction.

2.4. Statistics

The results are presented as percentages of relaxation. Cumulative concentration–response relationships for the vasorelaxant effects of hydroxylamine or sodium nitroprusside were analyzed with non-linear curve fitting by means of a logistic equation (Grafit, Erithacus Software) and IC_{50} values were obtained as the drug concentration causing a half-maximum relaxation. Results are expressed as the means $\pm$ S.E.M. for n separate experiments. Data were analyzed by Student's t-test and a probability of less than 0.05 was regarded significant.

3. Results

3.1. Role of endothelium in hydroxylamine-induced relaxation

Phenylephrine at 0.1 μM (approximate EC_{80} concentration causing a 80% of the maximum contraction) induced a sustained contraction (7.40 ± 0.62 mN, $n = 8$) of rat isolated aortic rings in the presence of endothelium. The trace in Fig. 1a shows that cumulative application of hydroxylamine (0.01–10 μM) caused a reduction of the sustained tension in a concentration-dependent manner (IC_{50}: 0.45 ± 0.10 μM, $n = 8$, Fig. 1b). Removal of the endothelium increased the phenylephrine-induced tone (12.50 ± 0.55 mN, $n = 25$) but did not affect the relaxant response to hydroxylamine (IC_{50}: 0.37 ± 0.06 μM, $n = 25$, in the absence of endothelium, Fig. 1b). In addition, pretreatment with L-NOARG (100 μM) did not influence the hydroxylamine-induced relaxation (IC_{50}: 0.49 ± 0.10 μM, $n = 7$, Fig. 1b), but this concentration of L-NOARG completely abolished the maximal relaxation induced by 1 μM acetylcholine in the endothelium-intact arteries ($n = 5$, data not shown). In the endothelium-denuded aortic rings, the concentration–relaxation curve for the relaxant effect of hydroxylamine was markedly shifted to the right in the presence of 10 μM methylene blue (IC_{50}: 107.2 ± 9.2 μM, $n = 5$, $P < 0.05$ compared with the control, Fig. 1b). In the time-matched control experiments, the phenylephrine-induced steady tension was reduced by approximately 4% in Krebs solution when measured 90 min after addition of phenylephrine ($n = 5$), indicating that the sustained contraction could be maintained for the time needed to construct a complete concentration–response curve for the relaxant effect of hydroxylamine.

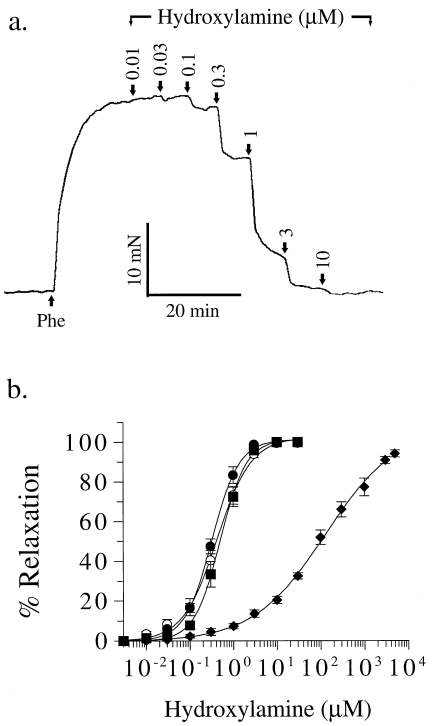

Fig. 1. The trace (a) is a representative record of the relaxant effects of hydroxylamine in an artery with endothelium. (b) The relaxant effect of hydroxylamine on phenylephrine (0.1 μM)-contracted rat aortic rings in the presence (○, $n = 8$) and absence (●, $n = 25$) of the endothelium, the effect of N^G-nitro-L-arginine (100 μM) on the relaxation induced by hydroxylamine in endothelium-intact arteries (■, $n = 7$) and the effect of methylene blue (10 μM) on the relaxation induced by hydroxylamine in endothelium-denuded arteries (◆, $n = 5$). Tissues were pretreated with each drug for 30 min prior to application of phenylephrine. Data are means ± S.E.M. of n experiments.

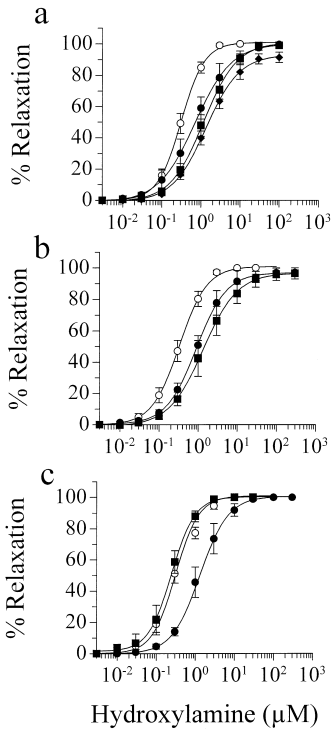

Fig. 2. Effects of K^+ channel blockers on the relaxation induced by hydroxylamine in rat aortic rings in the absence of endothelium. (a) The effect of tetrapentylammonium ions (TPA^+) on hydroxylamine-induced relaxation (control: ○, $n = 11$; 0.3 μM TPA^+: ●, $n = 7$; 1 μM TPA^+: ■, $n = 7$; 3 μM TPA^+: ◆, $n = 7$). (b) The effect of tetraethylammonium ions (TEA^+) on hydroxylamine-induced relaxation (control: ○, $n = 7$; 1 mM TEA^+: ●, $n = 6$; 3 mM TEA^+: ■, $n = 6$). (c) Effects of charybdotoxin and glibenclamide on hydroxylamine-induced relaxation (control: ○, $n = 6$; 100 nM charybdotoxin: ●, $n = 6$; 3 μM glibenclamide: ■, $n = 6$). Tissues were pretreated with each K^+ channel blocker for 30 min prior to application of phenylephrine (0.1 μM). Data are means ± S.E.M. of n experiments.

3.2. Effects of putative K^+ channels blockers on hydroxylamine-induced relaxation

The relaxant effect of hydroxylamine on induced tone was examined in endothelium-denuded arteries pretreated with various putative blockers of K_{Ca} channels, tetrapentylammonium ions, charybdotoxin and tetraethylammonium ions. Fig. 2a shows that tetrapentylammonium ions pro-

gressively inhibited the relaxation induced by hydroxylamine (IC_{50}: 0.36 ± 0.09 μM, $n = 11$; 1.29 ± 0.39 μM, $n = 7$; 1.47 ± 0.35 μM, $n = 7$; 1.63 ± 0.42 μM, $n = 7$ for 0, 0.3, 1 and 3 μM tetrapentylammonium ions; $P < 0.05$ compared with control, Table 1). Tetrapentylammonium ions at 3 μM reduced the hydroxylamine-induced maximal relaxation by 8.7 ± 3.3% ($n = 7$). In addition, pretreatment with tetraethylammonium or charybdotoxin also partially inhibited the relaxation induced by hydroxylamine (IC_{50}: 0.36 ± 0.04 μM, $n = 7$; 1.22 ± 0.35 μM, $n = 6$; 2.19 ± 0.39 μM, $n = 6$ for 0, 1 and 3 mM tetraethylammonium ions; $P < 0.05$ from the control, Fig. 2b, and IC_{50}: 0.35 ± 0.06 μM, $n = 6$ in the control; 1.82 ± 0.57 μM, $n = 6$ for 100 nM charybdotoxin, $P < 0.05$ from the control, Fig. 2c, Table 1). Combined pretreatment with tetrapentylammonium ions (3 μM) and charybdotoxin (100 nM) caused a more profound inhibition of the hydroxylamine-induced relaxation (IC_{50}: 4.92 ± 0.91 μM, $n = 6$) than did tetrapentylammonium ions or charybdotoxin alone (Fig. 3). Preincubation of the aortic rings with the K^+ channel blockers did not affect the magnitude of the phenylephrine-induced sustained tension (12.4 ± 0.8 mN, $n = 11$ for the control, 11.5 ± 0.9 mN, $n = 7$ for 3 μM tetrapentylammonium ions, 13.0 ± 2.0 mN, $n = 6$ for 3 mM tetraethylammonium ions, 14.1 ± 2.3 mN, $n = 6$ for 100 nM charybdotoxin). In contrast, glibenclamide at 3 μM did not alter the hydroxylamine-induced relaxation (IC_{50}: 0.31 ± 0.07 μM, $n = 6$, Fig. 2c) but this concentration completely reversed the relaxation induced by 3 μM pinacidil ($n = 4$, data not shown).

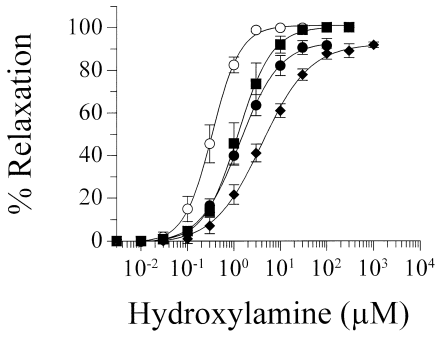

Fig. 3. The combined effect of tetrapentylammonium ions (TPA^+, 3 μM) and charybdotoxin (100 nM) on the relaxation induced by hydroxylamine in rat aortic rings in the absence of endothelium (control: ○, $n = 7$; TPA^+: ●, $n = 6$; charybdotoxin: ■, $n = 6$; 3 μM TPA^+ plus 100 nM charybdotoxin: ♦, $n = 6$). Data for TPA^+ or charybdotoxin are the same as in Fig. 2a or b. Tissues were preincubated with drugs for 30 min prior to application of phenylephrine (0.1 μM). Data are means ± S.E.M. of n experiments.

Table 1
Effects of K^+ channel blockers on the relaxation induced by nitric oxide donors

	Hydroxylamine			Sodium nitroprusside		
	IC_{50} (μM)	E_{max} (%)	n	IC_{50} (nM)	E_{max} (%)	n
TPA^+ (μM)						
0	0.36 ± 0.09	100	11	2.31 ± 0.36	100	12
0.3	1.29 ± 0.39[a]	99.8 ± 0.3	7			
1	1.47 ± 0.35[a]	99.1 ± 0.9	7			
3	1.63 ± 0.42[a]	91.3 ± 3.3	7	22.6 ± 3.89[a]	96.6 ± 2.0	6
TEA^+ (mM)						
0	0.36 ± 0.04	100	7			
1	1.22 ± 0.35[a]	97 ± 3.2	6			
3	2.19 ± 0.39[a]	96.4 ± 3.5	6	6.97 ± 0.98[a]	100	6
CTX (nM)						
0	0.35 ± 0.06	100	6			
100	1.82 ± 0.57[a]	100	6	4.53 ± 0.74[a]	100	6
CTX (100 nM) + TPA^+ (3 μM)						
	4.92 ± 0.91[a]	98.2 ± 1.3	6			
Glibenclamide (μM)						
3	0.31 ± 0.07	100	6	2.22 ± 0.23	100	5

The IC_{50} values and the maximum relaxation (E_{max}) induced by hydroxylamine and sodium nitroprusside in the absence and presence of putative K^+ channel blockers in the endothelium-denuded rat aorta. TPA^+, tetrapentylammonium ions; TEA^+, tetraethylammonium ions; CTX, charybdotoxin. Significant differences between the control and treatment groups ([a]$P < 0.05$) are indicated. Results are means ± S.E.M. from n experiments.

3.3. Effects of K^+ channel blockers on sodium nitroprusside-induced relaxation

Sodium nitroprusside, an exogenous nitric oxide donor, concentration dependently relaxed the endothelium-denuded aortic rings precontracted with 0.1 μM phenylephrine (IC_{50}: 2.31 ± 0.36 nM, $n = 12$). Fig. 4 shows that pretreatment of aortic tissues with tetrapentylammonium ions (3 μM), tetraethylammonium ions (3 mM) and charybdotoxin (100 nM) partially reduced the relaxation induced by sodium nitroprusside (IC_{50}: 22.6 ± 3.89 nM,

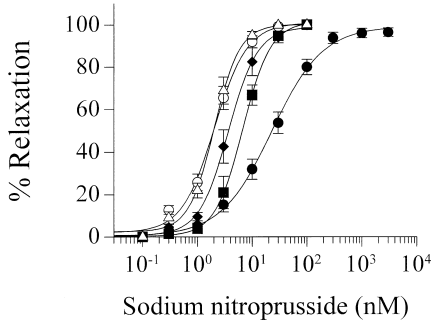

Fig. 4. Effects of K^+ channel blockers on the relaxation induced by sodium nitroprusside in rat aortic rings in the absence of endothelium (control: ○, $n = 12$; 3 mM tetraethylammonium ions: ■, $n = 6$; 100 nM charybdotoxin: ♦, $n = 6$; 3 μM tetrapentylammonium ions: ●, $n = 6$; 3 μM glibenclamide: △, $n = 5$). Tissues were pretreated with each K^+ channel blocker for 30 min prior to application of phenylephrine (0.1 μM). Data are means ± S.E.M. of n experiments.

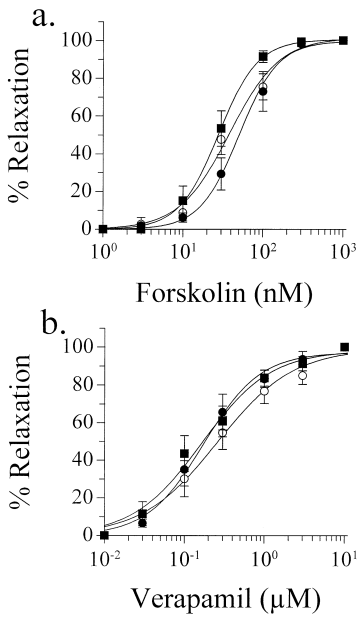

Fig. 5. Effects of K^+ channel blockers on relaxations induced by forskolin (a) and by verapamil (b) in rat aorta without endothelium. Symbols in (a and b): ○, $n = 6–7$ in control; ●, $n = 5$ in 3 μM tetrapentylammonium ions; ■, $n = 5$ in 3 mM tetraethylammonium ions. Tissues were pretreated with each K^+ channel blocker for 30 min prior to application of phenylephrine (0.1 μM). Data are means ± S.E.M. of n experiments.

$n = 6$ for tetrapentylammonium ions; 6.97 ± 0.98 nM, $n = 6$ for tetraethylammonium ions; 4.53 ± 0.74 nM, $n = 6$ for charybdotoxin; $P < 0.05$ compared with the control value, Table 1). These blockers of K_{Ca} channels did not affect the complete relaxation induced by sodium nitroprusside (Fig. 4). In contrast, glibenclamide (3 μM) had no effect (IC_{50}: 2.22 ± 0.23 nM, $n = 5$).

3.4. Effects of K^+ channel blockers on relaxations induced by forskolin and verapamil

In order to test the possibility that putative K^+ channel blockers may also inhibit the nitric oxide-independent relaxation in rat aorta, the relaxation induced by forskolin or verapamil was compared in the absence and presence of two quaternary ammonium compounds. Fig. 5 shows that pretreatment of the endothelium-denuded aortic tissues with either tetrapentylammonium ions (3 μM) or tetraethylammonium ions (3 mM) did not alter the relaxation induced by forskolin (IC_{50}: 38.1 ± 6.4 nM, $n = 7$ for control; 50.1 ± 6.4 nM, $n = 5$ for tetrapentylammonium ions; 29.7 ± 4.6 nM, $n = 5$ for tetraethylammonium ions, $P > 0.05$ in comparison with control, Fig. 5a). Similarly, putative K^+ channel blockers did not influence the verapamil-induced relaxation (0.26 ± 0.05 μM, $n = 6$ for control; 0.18 ± 0.03 μM, $n = 5$ for tetrapentylammonium ions; 0.17 ± 0.04 μM, $n = 5$ for tetraethylammonium ions, $P > 0.05$ in comparison with control, Fig. 5b).

3.5. Effect of high extracellular K^+ levels on hydroxylamine-induced relaxation

Sustained contractions of similar magnitude were induced in the presence of three extracellular K^+ concentrations by phenylephrine at reduced concentrations (0.08 μM for 15 mM K^+; 0.03 μM for 30 mM K^+ and 0 μM for 60 mM K^+). Fig. 6a shows the effect of increasing extracellular K^+ concentration on the relaxation induced by hydroxylamine. The concentration–relaxation curve for hydroxylamine was progressively shifted to the right when extracellular K^+ was raised from 5.9 to 60 mM (IC_{50}: 0.37 ± 0.09 μM, $n = 8$ for the control; 1.26 ± 0.46 μM, $n = 5$ for 15 mM K^+; 1.80 ± 0.17 μM, $n = 5$ for 30 mM K^+ and 3.81 ± 0.53 μM, $n = 9$ for 60 mM K^+). In arteries contracted with 60 mM K^+, the maximal relaxation induced by hydroxylamine was reduced by 13 ± 4.3% ($n = 9$). In addition, neither tetrapentylammonium ions (3 μM) nor charybdotoxin (100 nM) affected the hydroxylamine-induced relaxation of the artery precontracted with 60 mM K^+ (IC_{50}: 3.39 ± 0.49 μM, $n = 5$ for tetrapentylammonium ions and 4.03 ± 0.48 μM for charybdotoxin, $P > 0.05$ compared with 3.36 ± 0.33 μM, $n = 5$ of the control, Fig. 6b).

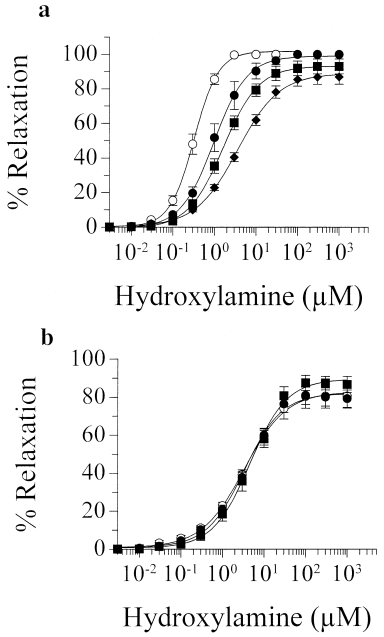

Fig. 6. The effect of high extracellular K^+ concentration on the relaxation induced by hydroxylamine (a) in rat aortic rings in the absence of endothelium. The artery was precontracted under different conditions and hydroxylamine induced concentration-dependent relaxation (○, $n = 8$, 0.1 μM phenylephrine in control; ●, $n = 5$, 0.08 μM phenylephrine in 15 mM K^+; ■, $n = 5$, 0.03 μM phenylephrine in 30 mM K^+; ♦, $n = 9$, 60 mM K^+). Lack of effect of tetrapentylammonium ions (TPA^+, 3 μM) or charybdotoxin (100 nM) on hydroxylamine-induced relaxation (b) in tissues contracted with 60 mM K^+ (○, $n = 5$ in control, ●, $n = 5$ in TPA^+, ■, $n = 5$ in charybdotoxin). Each K^+ channel blocker was added 30 min prior to application of 60 mM K^+. Data are means ± S.E.M. of n experiments.

4. Discussion

In aortic smooth muscle cells, K_{Ca} channels may contribute to maintenance of the membrane potential (Shoemaker and Worrel, 1991). More recently, charybdotoxin-sensitive K_{Ca} channels were found to be activated in association with myogenic tone in pressurized rabbit cerebral arteries (Brayden and Nelson, 1992), indicating that modulation of K_{Ca} channel activity might be an important mechanism that regulates the level of muscle contractility and vascular tone. The results of the present study suggest an interaction between the actions of nitric oxide donors and K_{Ca} channels in rat aortic smooth muscle. Activation of K_{Ca} channels contributes to the vasorelaxant effects of hydroxylamine and sodium nitroprusside. The hydroxylamine-induced relaxation in rat isolated aortic rings was inhibited by charybdotoxin, tetrapentylammonium ions or tetraethylammonium ions, putative blockers of large-conductance K_{Ca} channels (Huang and Nelson, 1990; Langton et al., 1991; Miller et al., 1985). Furthermore, charybdotoxin, tetrapentylammonium and tetraethylammonium ions also significantly reduced the relaxation induced by sodium nitroprusside, an exogenous nitric oxide donor. However, tetrapentylammonium ions (3 μM) were more effective than tetraethylammonium ions (3 mM) and charybdotoxin (100 nM) in inhibiting the sodium nitroprusside-induced response. In contrast, tetrapentylammonium and tetraethylammonium ions did not change relaxations induced by nitric oxide-independent vasodilators, forskolin and verapamil. Moreover, the forskolin-induced relaxation was unaltered by charybdotoxin in rat mesenteric artery (Huang and Kwok, 1997). These results indicate that the activation of K_{Ca} channels may be partially involved in the relaxation mediated by both endogenous and exogenous nitric oxide donors. The results of the present study are in agreement with the contention that nitric oxide probably activates vascular K_{Ca} channel either directly (Archer et al., 1994; Bialecki and Stinson-Fisher, 1995) and/or through cyclic GMP-dependent mechanisms (Robertson et al., 1993; Taniguchi et al., 1993; Williams et al., 1988). In the present study, tetrapentylammonium ions at the maximally effective concentration (3 μM) and charybdotoxin at 100 nM caused approximately 5-fold rightward shifts of the hydroxylamine concentration–response curve, respectively. Charybdotoxin when combined with tetrapentylammonium ions (3 μM) further attenuated the hydroxylamine-induced relaxation in the endothelium-denuded arteries (13-fold rightward shift). Charybdotoxin is a potent blocker of K_{Ca} channels in smooth muscle and in other preparations, with a K_d between 1 and 20 nM (Blatz and Magleby, 1987; Brayden and Nelson, 1992; Miller et al., 1985), while external tetrapentylammonium ions could inhibit large conductance K_{Ca} channels in rat arterial smooth muscle at higher concentrations ($K_D = 1.49$ mM) (Langton et al., 1991). However, much lower concentrations (0.3–3 μM) of tetrapentylammonium ions were needed to attenuate the nitric oxide donor-mediated relaxation in the present work. These results, therefore, indicate that different types of K^+ channels may be involved in the relaxation induced by hydroxylamine.

In vascular tissues, K_{ATP} channels are activated by various K^+ channel openers, and subsequent membrane hyperpolarization contributes to smooth muscle relaxation (Edwards and Weston, 1994; Nelson and Quayle, 1995; Standen et al., 1989). Activation of K_{ATP} channels and ensuing hyperpolarization in arterial smooth muscle are inhibited by glibenclamide, a sulfonylurea agent, but not by charybdotoxin (Nelson et al., 1990a; Standen et al., 1989). In the present experiments, glibenclamide had no effect on the relaxation induced by hydroxylamine or sodium nitroprusside. However, glibenclamide reversed the relaxation induced by pinacidil, an activator of K_{ATP} channels (Standen et al., 1989). These results suggest that the activation of K_{ATP} channels may not play a role in nitric oxide donor-mediated relaxation in the aortic preparation. The present results obtained with rat aorta are in contrast to the observation that nitric oxide induced hyperpolarization via glibenclamide-sensitive K_{ATP} channels in rat and rabbit mesenteric arteries (Garland and McPherson, 1992; Murphy and Brayden, 1995). In rabbit mesenteric artery, the hyperpolarization induced by nitric oxide released from 3-morpholinosydnonimine was not attenuated by various putative K_{Ca} channel blockers such as charybdotoxin, iberiotoxin, tetraethylammonium ions and apamin (Murphy and Brayden, 1995). Glibenclamide failed to change the endothelium-dependent hyperpolarization in some other arteries (Chen et al., 1991; Garland and McPherson, 1992). These findings imply that there is variation in the type of K^+ channels present in smooth muscle cells from different arteries, as proposed by Plane and Garland (Plane and Garland, 1993). In view of the activator effect of hydroxylamine and sodium nitroprusside on K_{ATP} channels in pancreatic β-cells (Antoine et al., 1996), the lack of effect of glibenclamide on the nitric oxide donor-mediated relaxation may indicate a variation in the characteristics of the glibenclamide-sensitive K^+ channels in different vessels and in their contribution to the membrane potential in the different cell types.

In many instances, the vasorelaxation induced by membrane hyperpolarization of arterial smooth muscle is caused by an increase in K^+ permeability, and so this possibility was tested by replacing external Na^+ with K^+. High extracellular K^+ would depolarize the cell membrane by reducing the electrochemical gradient for K^+ efflux. A rise in extracellular K^+ from 5.9 to 60 mM progressively diminished the relaxation induced by hydroxylamine. In addition, in arteries contracted with 60 mM K^+, the relaxation induced by hydroxylamine was not affected by tetrapentylammonium ions at 3 μM, a concentration that maximally attenuated the hydroxylamine-induced reduction in evoked tension, or by charybdotoxin. Similarly, the hyperpolarization induced by 3-morpholinosydnonimine

was attenuated by an elevation of extracellular K^+ from 6 to 12 mM in rabbit mesenteric artery (Murphy and Brayden, 1995). These findings further suggest that the activation of K^+ channels involves the vascular responses to hydroxylamine and probably also to exogenous nitric oxide donors.

The relaxant effect of hydroxylamine in the phenylephrine-contracted rat aorta was independent of the endothelium. In addition, L-NOARG, an inhibitor of endothelial nitric oxide synthase, did not attenuate the arterial response to hydroxylamine. L-NOARG at 100 μM caused a complete inhibition of the acetylcholine-induced endothelium-dependent relaxation in the same tissues. These results are consistent with those of the previous report of Thomas and Ramwell (1989). Methylene blue, an inhibitor of soluble gunanylate cyclase, reduced the hydroxylamine-induced relaxation in the endothelium-denuded arteries by approximately 260-fold, suggesting that hydroxylamine exerts its action primarily by releasing nitric oxide, which may increase intracellular cyclic GMP levels in arterial smooth muscle to mediate vasorelaxation.

Taken together, the present results indicate that the endothelium-independent relaxation induced by hydroxylamine and sodium nitroprusside in rat isolated aortic rings is partially mediated by the activation of K^+ channels which are sensitive to inhibition by charybdotoxin, tetrapentylammonium and tetraethylammonium ions. It is possible that nitric oxide released from both endogenous and exogenous nitric oxide donors may activate more than one type of K^+ channel in arterial smooth muscle cells.

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