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Comparison of the vascular relaxant effects of ATP-dependent K^+ channel openers on aorta and pulmonary artery isolated from spontaneously hypertensive and Wistar–Kyoto rats

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Abstract

The vasorelaxant actions of adenosine 5′-triphosphate (ATP)-dependent K^+ channel openers and sodium nitroprusside in isolated thoracic aorta and pulmonary artery of spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats (14–18 weeks old) were investigated. Cumulative addition of sodium nitroprusside and different ATP-dependent K^+ channel openers (pinacidil, cromakalim, nicorandil, 2-(2″(1″,3″-dioxolone)-2-methyl-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2*H*-1-benzopyren (KR-30450) and aprikalim) to these preparations caused a concentration-dependent relaxation of noradrenaline-pre-contracted aorta and pulmonary artery from both strains. The relative order of relaxation potency, estimated by comparing the IC_{50}, was sodium nitroprusside > KR-30450 > aprikalim ≥ cromakalim > pinacidil > nicorandil in pulmonary artery and aorta from both strains. At high concentrations (≥ 1 μM), cromakalim, aprikalim and KR-30450 produced a greater percentage relaxation in SHR aorta than in WKY aorta. However, there was no apparent difference between SHR and WKY in the relaxation response to all drugs tested on the pulmonary artery. The effects of cromakalim, aprikalim, pinacidil and KR-30450 observed in aorta and pulmonary artery were significantly attenuated by 3 μM glibenclamide. 6-Anilino-5,8-quinolinequinone (LY 83583, 1 μM), a soluble guanylate cyclase inhibitor, abolished the vasorelaxant effects of nicorandil and sodium nitroprusside. In conclusion, sodium nitroprusside and ATP-dependent K^+ channel openers cause relaxation of noradrenaline-pre-contracted aorta and pulmonary artery from both strains. However, all the drugs tested failed to cause selective relaxation of the pulmonary artery relative to the thoracic aorta.

Keywords: ATP-sensitive K^+ channel; Spontaneously hypertensive rat (SHR); Pulmonary artery; Thoracic aorta

1. Introduction

The most common causes of pulmonary arterial hypertension are heart disease and lung disease. The aim of pharmacological therapy for pulmonary hypertension is to induce pulmonary vasodilatation, thereby elevating systemic blood flow, and to reduce the workload of the right ventricle. However, no single vasodilator has been demonstrated to successfully achieve this goal (Daoud et al., 1978; Ruskin and Hutter, 1979; Rubin and Peter, 1980; Camerini et al., 1980; Palevsky and Fishman, 1985).

Since the first description of the adenosine 5′-triphosphate (ATP)-sensitive K^+ channels (K_{ATP}) in cardiac myocytes (Noma, 1983; Noma and Shibasaki, 1985), these channels have been identified in other tissues including vascular smooth muscle cells (Standen et al., 1989). The K_{ATP} channels are specifically blocked by sulfonylurea derivatives such as tolbutamide and glibenclamide (Aschroft, 1990; Hamada et al., 1990) and are activated by a number of ATP-dependent K^+ channel openers (Sanguinetti et al., 1988; Hiraoka and Fan, 1989; Arena and Kass, 1989; Thuringer et al., 1995; Kwak et al., 1995). The opening of ATP-sensitive K^+ channels results in hyperpolarization of the plasma membrane (for review see Longman and Hamilton, 1992) and hence, in vasodilatation of vascular smooth muscle. It is therefore possible that these ATP-sensitive K^+ channel openers can be used in the treatment of systemic hypertension and angina pectoris (Quast, 1992; Di Somma et al., 1993).

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In most studies examining the vasorelaxant (anti-hypertensive) effects of different compounds, only the isolated aorta of spontaneously hypertensive rats (SHR) was used (Spector et al., 1969; Clineschmidt et al., 1970). However, there is evidence to suggest that various vasculatures of the same animal react differently to drugs (Altura, 1974; Magnon et al., 1994). Furthermore, a systematic comparison of the vascular relaxant effects of different ATP-sensitive K^+ channel openers on pulmonary artery and thoracic aorta has not been examined in detail especially under diseased conditions.

In most studies, experimental pulmonary hypertension is usually induced by either chronic hypoxia or monocrotaline (Wanstall and O'Donnell, 1992; Crawley et al., 1992; Wanstall et al., 1993). Genetically developed hypertensive rats (spontaneously hypertensive rats, SHR) have mostly been used to provide isolated thoracic aorta (Spector et al., 1969; Clineschmidt et al., 1970) but not pulmonary artery preparations for various drug studies. Genetic factors, however, are also important determinants of the development of hypertension. It is therefore of utmost importance to examine how the vascular relaxant effects of ATP-dependent K^+ channel openers are modified by genetically developed hypertension of the pulmonary circulation (Aharinejad et al., 1996).

Hence, the first aim of this study is to investigate and compare the vascular relaxant effects of different ATP-sensitive K^+ channel openers in noradrenaline-pre-contracted thoracic aorta and pulmonary artery isolated from normotensive Wistar–Kyoto (WKY) rats, and to determine whether there is a selective relaxation in pulmonary artery relative to thoracic aorta. The second aim is to determine how hypertension affects the vascular responses to different ATP-sensitive K^+ channel openers of thoracic aorta and pulmonary artery obtained from spontaneously hypertensive (SHR) rats.

2. Materials and methods

2.1. Animals

The experiments were approved by The Animal Ethics Committee of the Chinese University of Hong Kong. The spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats were bred in our own colony from three pairs (three SHR and three WKY rats) purchased from the Animal Resources Centre (Australia). The SHR and the normotensive WKY rats (male) were 14–18 weeks old and weighed 325 ± 13 g and 341 ± 18 g, respectively. Rats were housed under a 12:12-h light–dark cycle and were given standard rat chow and water ad libitum before they were killed.

2.2. Tissue preparations

Rats were killed by cervical dislocation. The thoracic aorta and primary pulmonary artery were isolated and excess fat and connective tissue were removed. Care was taken to prevent damage to the endothelium, and the presence of functional endothelium was confirmed by 10 μM acetylcholine-induced relaxation of 40 mM $[K^+]_o$-pre-contracted tissue preparations at the beginning of each experiment. Aortae were cut into rings of about ~3 mm and pulmonary arteries into rings of ~2 mm in length and mounted in a 5-ml vertical organ bath containing Krebs' solution of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaCl_2$ 2.5, propranolol 0.001 and indomethacin 0.001. The bath solution was gassed with 95% O_2–5% CO_2 (pH 7.4, 37 ± 1°C).

Two stainless steel hooks were inserted into the lumen of the isolated blood vessel; one was fixed and the other was connected to a force-displacement transducer (Grass FT 03). Isometric tension measurement was performed by using the MacLab Chart v 3.4 program. Data were stored on the hard disk of a MacIntosh computer for subsequent analysis. The aorta and pulmonary artery rings were equilibrated under a resting tension of 1.5 ± 0.2 g and 1.0 ± 0.1 g, respectively, in the bath solution for 90 min. During the equilibration period, the preparation was washed with drug-free Krebs' solution every 20 min and the resting tension was readjusted, if necessary, before the experiments started.

After equilibration, isolated thoracic aorta and pulmonary artery were sensitized with 40 mM KCl. This sensitization process was considered complete when two consecutive contractile responses to high $[K^+]_o$ were reproducible. To investigate the relaxant effects of different drugs in isolated pulmonary artery and thoracic aorta, the preparations were pre-contracted with 0.3 μM noradrenaline, a concentration which is the approximate EC_{85} observed in pulmonary artery and aorta of both SHR and WKY rats ($n = 6$ for each preparation) (in the presence of 1 μM propranolol). After a steady-state contraction was established, cumulative concentrations (1 nM–100 μM) of drug were added to the organ bath. A time-matched control was used throughout the experiment and any change in tension observed was used for the subsequent adjustment of the drug-induced relaxation. Due to the lipophilicity of all the drugs examined in this study, only one concentration–response curve was obtained for each tissue preparation. The presence of the vehicles, dimethyl sulphoxide (DMSO) and absolute ethanol (0.5% vol/vol, the final concentration present in the organ bath), had no apparent effect on the noradrenaline-induced contraction of pulmonary artery and aorta from both strains ($n = 3$) or on the drug-mediated relaxation ($n = 3$ for each compound) (data not shown).

2.3. Drugs

Sodium nitroprusside dihydrate (dissolved in Nanopure water), (±)-*trans*-6-cyano-3,4-dihydro-2,2-dimethyl-4-[2-

oxopyrrolidin-1-yl]-2 *H*-1-benzopyran-3-ol (cromakalim) (dissolved in dimethyl sulphoxide), glibenclamide (dissolved in dimethyl sulphoxide), apamin (dissolved in Nanopure water), charybdotoxin (dissolved in Nanopure water) and N^G-nitro-L-arginine methyl ester (L-NAME) (dissolved in dimethyl sulphoxide) were obtained from Sigma (USA). *Trans*-(−)-*N*-methyl-2-(3-pyridyl)-2-tetrahydro-thio-pyran carbothiamide-1-oxide (aprikalim) (dissolved in absolute ethanol) was a gift from Rhône-Poulenc Rorer, France, and 2-nicotinamidoethyl nitrate (nicorandil) (dissolved in dimethyl sulphoxide) was a gift from Chugai Pharmaceuticals, Japan. (±)-*N*-cyano-4-pyridyl-*N*-1,2,2-trimethylpropylguanidine monohydrate (pinacidil) (dissolved in dimethyl sulphoxide) was obtained from Research Biochemicals International, USA. 2-(2″(1″,3″-dioxolone)-2-methyl-4-(2′-oxo-1′-pyrrolidinyl)-6-nitro-2 *H*-1-benzopyren (KR-30450) (dissolved in dimethyl sulphoxide) was a gift from The Korean Research Institute of Chemical Technology, Dae-Jeon, South Korea and 6-anilino-5,8-quinolinequinone (LY 83583) (dissolved in dimethyl sulphoxide) was purchased from Calbiochem-Novabiochem International (USA).

2.4. Data and statistical analysis

Change in tension, if any, was recorded and 100% relaxation was considered when the tension returned to baseline resting tension level. The IC_{50} and maximum response were estimated for individual concentration–response curves by use of non-linear least-squares regression (Prism 2.1, Graphpad, USA). Negative logarithmic values of IC_{50} were used for statistical analysis, although for ease of comprehension IC_{50} values are given in the text. All other values are given as means ± S.E.M.; when it is not visible, the standard error falls within the size of the symbol. Data were compared by using unpaired Student's *t*-test or analysis of variance (ANOVA), where appropriate. Differences were considered significant at $P < 0.05$ value.

3. Results

3.1. Effect of potassium and noradrenaline on thoracic aorta and pulmonary artery isolated from SHR and WKY rats

In the preliminary study, cumulative application of high $[K^+]_o$ evoked a concentration-dependent (10 mM–50 mM) contraction of aorta and pulmonary artery from SHR and WKY rats and 50 mM $[K^+]_o$ produced the maximum contraction in these preparations ($n = 4$ for each preparation) (data not shown). Thus, 50 mM $[K^+]_o$ was used as the reference for normalization of the noradrenaline-induced contraction.

In order to determine the concentration of noradrenaline to be used as contractile agent, cumulative concentration–response curves for noradrenaline in isolated thoracic aorta and pulmonary artery rings were generated and compared. Under resting tension, cumulative application of noradrenaline induced a concentration-dependent contraction of thoracic aorta and pulmonary artery rings from both SHR and WKY rats ($n = 5–6$ for each tissue preparation) (data not shown). Noradrenaline at 0.3 μM caused ~80–85% of the observed maximum contraction elicited by 50 mM $[K^+]_o$ in thoracic aorta and pulmonary artery rings from both SHR and WKY rats and produced a sustained contraction throughout the duration of the experiment (~160 min). This concentration of noradrenaline was therefore used in the subsequent relaxation study with different ATP-sensitive K^+ channel openers and sodium nitroprusside.

3.2. Effects of sodium nitroprusside on thoracic aorta and pulmonary artery isolated from SHR and WKY rats

Cumulative application of sodium nitroprusside (3 nM–1 μM) caused a concentration-dependent relaxation of 0.3 μM noradrenaline-pre-contracted aorta from SHR

Table 1
Summary of the vasorelaxant effects of sodium nitroprusside and different ATP-sensitive K^+ channel openers on noradrenaline-pre-contracted aorta from SHR and WKY rats

	Sodium nitroprusside	Aprikalim	Cromakalim	Nicorandil	KR-30450	Pinacidil
WKY						
IC_{50} (μM)	0.03 ± 0.01	0.56 ± 0.07	0.92 ± 0.09	13.91 ± 2.20	0.08 ± 0.03	3.17 ± 0.78
Maximum relaxation (%)	107.41 ± 1.62	75.05 ± 7.66	79.01 ± 4.30	97.41 ± 5.01	84.37 ± 4.91	105.85 ± 3.63
SHR						
IC_{50} (μM)	0.01 ± 0.01	0.29 ± 0.08[a]	0.53 ± 0.11	5.59 ± 0.83	0.07 ± 0.03	1.94 ± 0.82
Maximum relaxation (%)	121.37 ± 6.98	128.05 ± 15.17[b]	131.04 ± 14.37[a]	114.50 ± 2.95	110.02 ± 13.61	123.02 ± 7.82

Results are expressed as means ± S.E.M. ($n = 5–7$).
[a] $P < 0.05$ compared to normotensive WKY rats.
[b] $P < 0.01$ compared to normotensive WKY rats.

and WKY rats with no apparent difference in the maximum relaxation ($n = 5–7$) ($P > 0.05$) (Table 1). As in the aortic preparations, sodium nitroprusside elicited a concentration-dependent relaxation of the pre-contracted pulmonary artery of SHR and WKY rats ($n = 5–7$) ($P > 0.05$) (Table 1). There was no apparent difference in the maximum relaxation induced by sodium nitroprusside in pulmonary artery from SHR and WKY rats ($n = 5–7$) (Table 1).

3.3. Effects of ATP-dependent K^+ channel openers on thoracic aorta and pulmonary artery isolated from SHR and WKY rats

3.3.1. KR-30450

As with sodium nitroprusside, cumulative administration of KR-30450 (10 nM–10 μM) caused a concentration-dependent inhibition of the pre-contracted aorta of

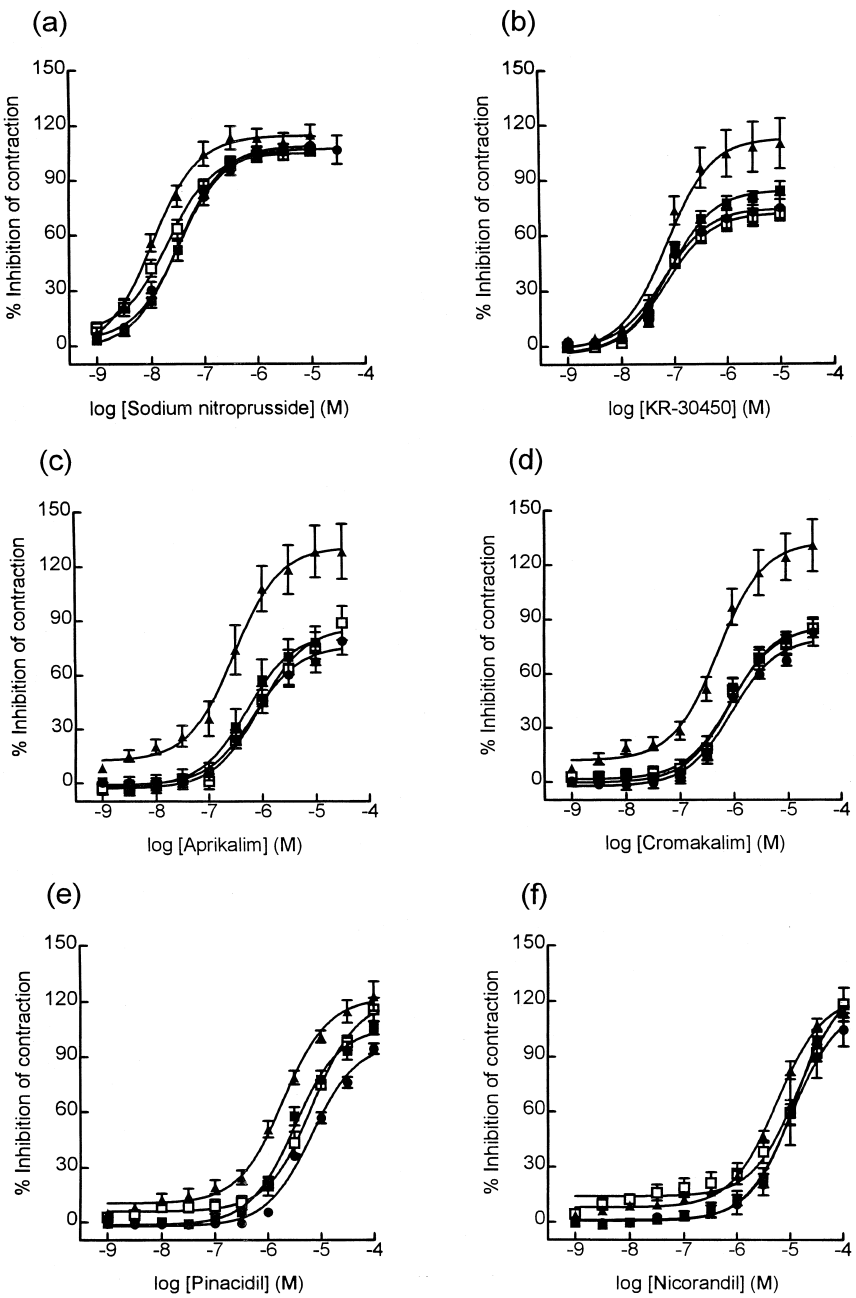

Fig. 1. Vascular relaxant responses of SHR thoracic aorta (▲) and pulmonary artery (□) and WKY thoracic aorta (■) and pulmonary artery (●) to increasing cumulative concentrations of (a) sodium nitroprusside, (b) KR-30450, (c) aprikalim, (d) cromakalim, (e) pinacidil and (f) nicorandil. Isolated thoracic aorta and pulmonary artery preparations were pre-contracted by 0.3 μM noradrenaline. Data points are means ± S.E.M. ($n = 5–7$).

SHR and WKY rats ($P > 0.05$) ($n = 5–7$) (Table 1). In contrast to the responses to sodium nitroprusside, the maximum relaxation induced by KR-30450 in SHR aorta was significantly greater than that observed in WKY aorta ($n = 5–7$) ($P < 0.05$) (Fig. 1 and Table 1). Application of KR-30450 also produced a concentration-dependent inhibition of the pre-contracted pulmonary artery of SHR and WKY rats ($P > 0.05$) and there was no apparent difference in the maximum relaxation between two strains ($n = 5–7$) (Table 1).

3.3.2. Aprikalim

Administration of aprikalim produced a significantly greater percentage inhibition of contraction, in a concentration-dependent fashion (10 nM–30 μM), in the pre-contracted aorta of SHR than in the aorta of WKY rats ($P < 0.05$) ($n = 5–7$) (Table 1). The maximum relaxation observed in SHR aorta was significantly greater than that observed in WKY aorta (Fig. 1 and Table 1). In contrast to the aorta preparations, there was no apparent difference in the relaxant responses elicited by aprikalim (100 nM–30 μM) in pulmonary artery from SHR and WKY rats ($n = 5–7$) (Table 1). As with KR-30450, a significantly greater percentage maximum inhibition of contraction was obtained with aprikalim in SHR aorta ($P < 0.05$) (Fig. 1).

3.3.3. Cromakalim

Cumulative application of cromakalim (10 nM–30 μM) caused a concentration-dependent relaxation of the pre-contracted aorta of SHR and WKY rats. There was no significant difference in the observed IC_{50} value of cromakalim-mediated responses in SHR and WKY rats ($n = 5–7$) (Table 1). However, a greater maximum percentage inhibition of contraction was observed in SHR aorta than in WKY aorta (Fig. 1 and Table 1). Like aprikalim, cromakalim (100 nM–30 μM) caused a similar percentage inhibition of contraction in pulmonary artery from SHR and WKY rats (Fig. 1 and Table 2).

3.3.4. Pinacidil

Like the other three ATP-dependent K^+ channel openers (KR-30450, aprikalim, and cromakalim) studied, pinacidil (100 nM–100 μM) elicited a concentration-dependent relaxation of the pre-contracted aorta of SHR and WKY rats. There was no apparent difference between the vasorelaxant responses of pinacidil observed in both strains ($n = 5–7$) (Table 1). Besides, the vasorelaxant responses to pinacidil observed in the pulmonary artery of SHR and WKY rats were similar ($n = 5–7$) (Table 2). There was a non-significant trend for a greater maximum percentage relaxation induced by pinacidil in SHR aorta (Fig. 1).

3.3.5. Nicorandil

Cumulative administration of nicorandil (1 μM–100 μM) caused a concentration-dependent relaxation of the pre-contracted aorta and pulmonary artery of SHR and WKY rats ($n = 5–7$) (Tables 1 and 2). Like sodium nitroprusside, nicorandil was equally potent in relaxing the aorta and pulmonary artery of SHR and WKY rats and no apparent difference in the percentage relaxation was recorded ($n = 5–7$) (Fig. 1).

3.4. Effects of glibenclamide on the vasorelaxant responses to sodium nitroprusside and ATP-sensitive K^+ channel openers

The effect of the specific ATP-sensitive K^+ channel inhibitor, glibenclamide, on the vasorelaxant responses to sodium nitroprusside and ATP-sensitive K^+ channel openers was examined. Pre-incubation of the aorta and pulmonary artery with glibenclamide (3 μM) had no apparent effect on the resting tension or the noradrenaline-induced contraction ($n = 5–6$ for each type of tissue of both strains) (data not shown).

The presence of glibenclamide significantly attenuated the vasorelaxant responses elicited by KR-30450, cromakalim, aprikalim and pinacidil in aorta and pulmonary artery isolated from SHR and WKY rats. A rightward shift of the concentration–response curve of KR-30450, cromakalim, aprikalim and pinacidil was observed in the presence of glibenclamide. Fig. 2 shows the concentration–response curves of KR-30450, cromakalim, aprikalim and pinacidil measured in WKY aorta in the presence of 3 μM glibenclamide. A similar rightward shift of the concentration–response curves was recorded in WKY pulmonary artery as well as SHR aorta and pulmonary artery ($n = 5–6$ for each drug) (data not shown). However, the presence of glibenclamide (3 μM) did not have a signifi-

Table 2
Summary of the vasorelaxant effects of sodium nitroprusside and different ATP-sensitive K^+ channel openers on noradrenaline-pre-contracted pulmonary artery from SHR and WKY rats

	Sodium nitroprusside	Aprikalim	Cromakalim	Nicorandil	KR-30450	Pinacidil
WKY						
IC_{50} (μM)	0.03 ± 0.02	0.68 ± 0.11	0.93 ± 0.08	10.85 ± 1.20	0.06 ± 0.02	6.62 ± 0.73
Maximum relaxation (%)	106.66 ± 7.84	79.07 ± 7.43	83.39 ± 7.83	104.91 ± 8.99	74.69 ± 6.86	94.51 ± 2.90
SHR						
IC_{50} (μM)	0.02 ± 0.01	0.88 ± 0.06	0.90 ± 0.13	14.55 ± 1.23	0.07 ± 0.04	6.86 ± 1.12
Maximum relaxation (%)	105.95 ± 1.67	89.09 ± 9.38	85.29 ± 5.04	118.57 ± 8.99	71.58 ± 3.66	115.77 ± 6.32

Results are expressed as means ± S.E.M. ($n = 5–7$).

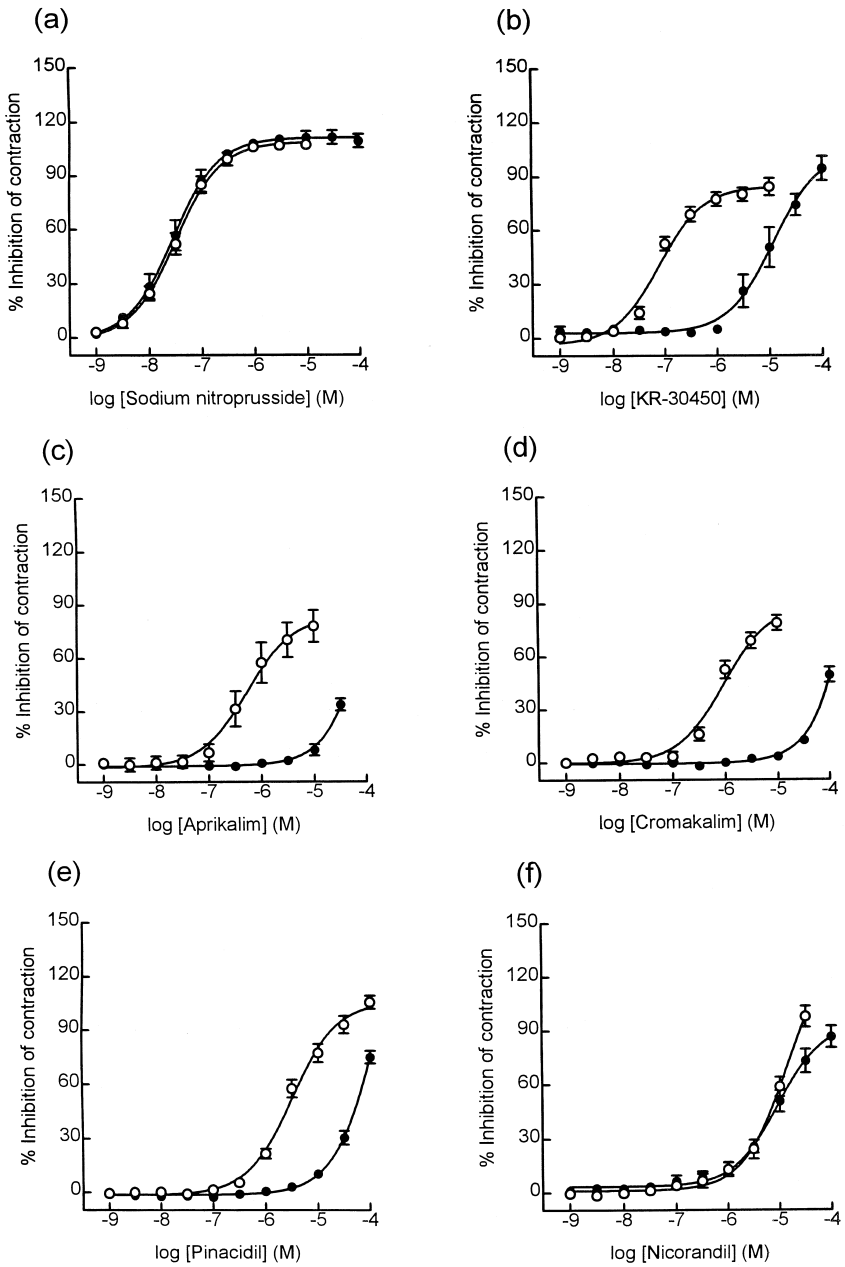

Fig. 2. Effect of glibenclamide on the vascular relaxant responses of WKY thoracic aorta to increasing cumulative concentrations of (a) sodium nitroprusside, (b) KR-30450, (c) aprikalim, (d) cromakalim, (e) pinacidil and (f) nicorandil. (○): Control and (●) in the presence of 3 μM glibenclamide. Isolated thoracic aorta were pre-contracted by 0.3 μM noradrenaline. Data points are means ± S.E.M. ($n = 5–7$).

cant effect on the nicorandil- and sodium nitroprusside-mediated relaxation observed in SHR and WKY aorta (Fig. 2) ($n = 5–6$) and pulmonary artery.

3.5. Effects of the Ca^{2+}-dependent K^+ channel inhibitors apamin and charybdotoxin and a nitric oxide (NO) synthase inhibitor L-NAME on the vasorelaxant responses to sodium nitroprusside and ATP-dependent K^+ channel openers

To investigate the role(s) of Ca^{2+}-dependent K^+ channels in the observed vasorelaxant effects of sodium nitroprusside and ATP-sensitive K^+ channel openers, the effects of two Ca^{2+}-dependent K^+ channel inhibitors (apamin and charybdotoxin) were studied. Application of apamin (1 μM) and charybdotoxin (100 nM) had no apparent effect on the resting tension and the noradrenaline-induced contraction in aorta and pulmonary artery from SHR and WKY rats (data not shown). The presence of L-NAME (20 μM) caused an approximate 32% increase in the noradrenaline-induced contractile response in all tissue preparations.

However, pre-incubation of the aorta and pulmonary artery from both strains with apamin (1 μM), charybdotoxin (100 nM) and L-NAME (20 μM) had no apparent

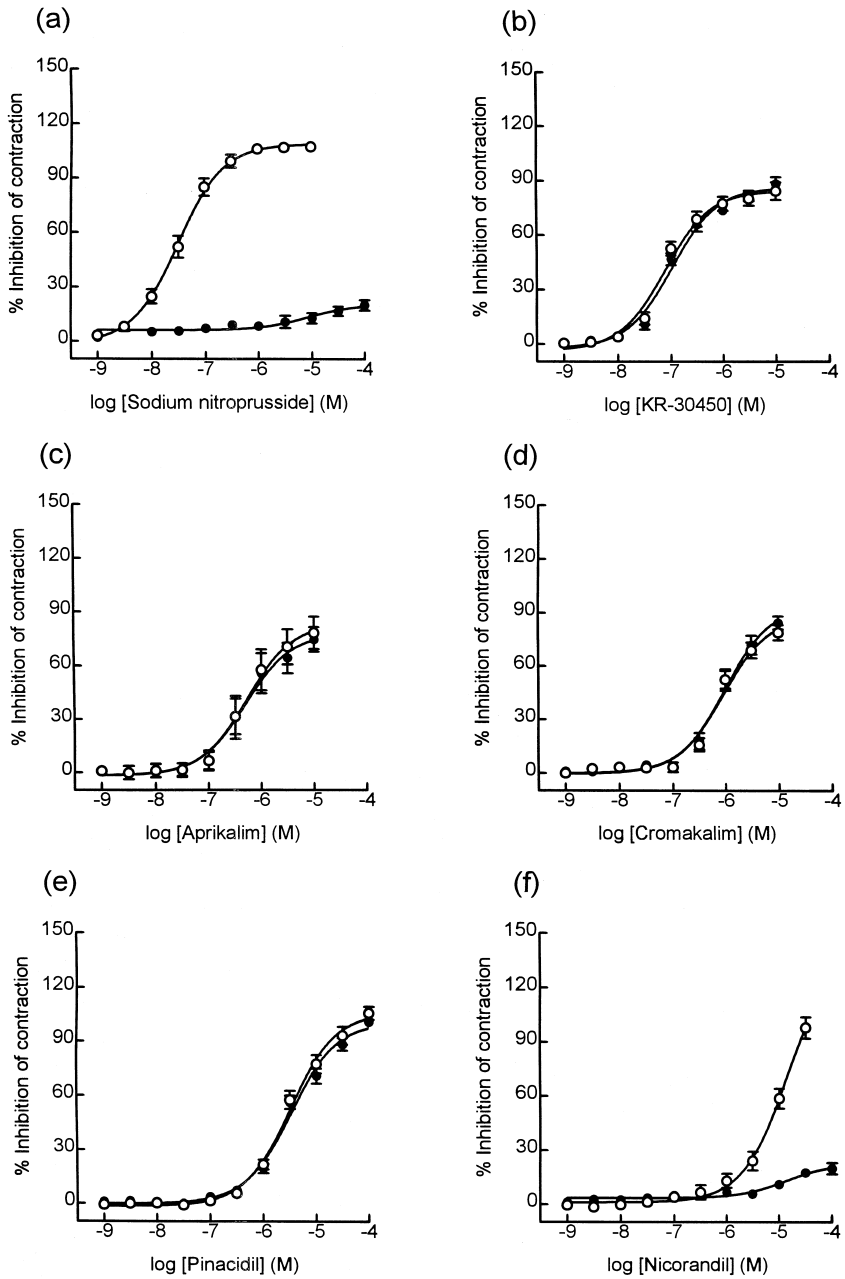

Fig. 3. Effect of LY 83583 (a guanylate cyclase inhibitor) on the vascular relaxant responses of WKY thoracic aorta to increasing cumulative concentrations of (a) sodium nitroprusside, (b) KR-30450, (c) aprikalim, (d) cromakalim, (e) pinacidil and (f) nicorandil. (○): Control and (●) in the presence of 1 μM LY 83583. Isolated thoracic aorta were pre-contracted by 0.3 μM noradrenaline. Data points are means ± S.E.M. ($n = 5–7$).

effect on the vasorelaxation elicited by sodium nitroprusside and ATP-sensitive K^+ channel openers ($n = 5–6$ for each drug in each type of tissue preparation) (data not shown).

3.6. Effects of a soluble guanylate cyclase inhibitor LY 83583 on the vasorelaxant responses to sodium nitroprusside and ATP-dependent K^+ channel openers

The presence of LY 83583 (1 μM) abolished the sodium nitroprusside- and nicorandil-elicited relaxation observed in the pre-contracted aorta (Fig. 3) and pulmonary artery of SHR ($n = 6$ for each drug) and WKY ($n = 7$ for each drug) rats ($P < 0.001$). However, the vasorelaxant effects of cromakalim, aprikalim, pinacidil and KR-30450 observed in pulmonary artery and aorta (Fig. 3) from SHR and WKY rats were not affected ($n = 5–7$ for each drug) ($P > 0.05$).

4. Discussion

We investigated the vascular relaxant effects of sodium nitroprusside (a standard guanylate cyclase activator) and different adenosine 5′-triphosphate (ATP)-dependent K^+

channel openers on thoracic aorta and pulmonary artery isolated from SHR and age-matched (14–18 weeks old) normotensive WKY rats (Aharinejad et al., 1996). It has been demonstrated that the vascular effects of some drugs/neurotransmitters are different in thoracic aorta isolated from SHR and normotensive WKY rats (Lograno et al., 1989). These findings indicate that under certain pathological conditions, e.g., hypertension, the responsiveness of the vasculature to different agents may be affected. In the present study, the contractile responses to noradrenaline of aorta and pulmonary artery from SHR were greater than the responses observed in the corresponding WKY preparations (Lograno et al., 1989; Hüsken et al., 1994).

In this study, application of different ATP-sensitive K^+ channel openers (KR-30450, aprikalim, cromakalim, pinacidil and nicorandil) and sodium nitroprusside (a guanylate cyclase activator) caused a concentration-dependent relaxation of noradrenaline-pre-contracted thoracic aorta and pulmonary artery from both SHR and WKY rats. The relative order of the inhibitory potency, determined by comparing the IC_{50} values, was sodium nitroprusside > KR-30450 > aprikalim ≥ cromakalim > pinacidil > nicorandil in WKY aorta (Table 1). A similar relative order of inhibitory potency was observed in WKY pulmonary artery (Table 2) and in SHR aorta and pulmonary artery (Tables 1 and 2). These results were in contrast to the results for Sprague–Dawley rats (Greenwood and Weston, 1993), in which cromakalim was found to be more potent than aprikalim in relaxing 20 mM $[K^+]_o$-pre-contracted isolated aorta. The discrepancy may be due to the species difference and/or the nature of the spasmogen used (Magnon et al., 1994).

In rabbits, Magnon et al. (1994) reported that aprikalim was more potent than nicorandil in relaxing noradrenaline-pre-contracted isolated pulmonary artery and aorta. Our present findings (Tables 1 and 2) are consistent with these results. However, in contrast to our results in which sodium nitroprusside was the most potent agent, the nitroglycerin-elicited relaxation observed in rabbit isolated pulmonary artery and aorta was smaller than the relaxation produced by aprikalim (Magnon et al., 1994). In addition, aprikalim and cromakalim (at higher concentrations of 1 μM to 30 μM) caused a significantly greater percentage inhibition of contraction in noradrenaline-pre-contracted SHR aorta (Fig. 1) than in SHR pulmonary artery or WKY aorta and pulmonary artery (Tables 1 and 2). It has been shown that the relaxant effects of cromakalim tend to be greater in carotid arteries from SHR than in arteries from WKY rats (Asano et al., 1994). Kashiwabara et al. (1994) reported that cromakalim caused relaxation of rat aorta with an IC_{50} value of ~0.14 μM. In our study, cromakalim caused relaxation of the pre-contracted aorta of WKY rats with an IC_{50} of 0.93 ± 0.08 μM.

Wanstall (1994) reported that pinacidil caused relaxation of pulmonary artery rings (pre-contracted by noradrenaline) with an IC_{50} of ~2.5 μM, which was fairly similar to the value observed in our study of 6.62 ± 0.73 μM in the pulmonary artery of WKY rats. In rats with pulmonary hypertension (induced either by chronic hypoxia or by injection of a plant toxin monocrotaline), a greater relaxation induced by pinacidil was seen only in pulmonary artery and not in aorta (Wanstall and O'Donnell, 1992; Wanstall et al., 1994). However, pinacidil elicited a similar degree of relaxation of the pre-contracted aorta and pulmonary artery of both strains in our study. The reason for the discrepancy is not known at present. Perhaps the underlying factors (e.g., genetic factors, chronic hypoxia and injection of monocrotaline) which are responsible for the development/induction of hypertension in rats may be important in determining the responses of the vasculature to drugs. Further experiments will be needed to clarify this point. Similar to the response to cromakalim and aprikalim, the percentage inhibition of the contraction elicited by KR-30450 (which occurred at higher concentrations) in SHR aorta was significantly greater than that observed in SHR pulmonary artery and in WKY aorta and pulmonary artery (Fig. 1).

The underlying mechanisms responsible for the difference between the relaxation induced by the ATP-dependent K^+ channel openers in preparations from SHR and WKY rats have not been fully elucidated so far. However, it is well-known that the ATP-sensitive K^+ channel openers consist of compounds with diverse chemical structures (Cook, 1988; Edwards and Weston, 1990). Activation of ATP-sensitive K^+ channels by these compounds depends on the intracellular concentrations of ATP, dinucleotide diphosphates, Mg^{2+} and H^+ ions (Findlay, 1987; Horie et al., 1987; Arena and Kass, 1989; Shen et al., 1991; Forestier et al., 1996). Altered signalling pathways have been demonstrated in cultured vascular smooth muscle cells from SHR (Tuttle et al., 1995) as well as altered properties of ATP-sensitive K^+ channels in mesenteric artery cells (Ohya et al., 1996) and in ventricular myocytes from diabetic rats (Smith and Wahler, 1996). Perhaps, in our study, hypertension caused a change in the sensitivity of the intracellular binding sites for ATP, dinucleotide diphosphates and Mg^{2+} ions as well as the drug-channel interactions.

In rats with pulmonary hypertension (induced by injection of monocrotaline), the relaxation responses to sodium nitroprusside and pinacidil in [5*Z*,9α,11α,13*E*,15*S*]-9,11,15-trihydroxyprosta-5,13-dienoic acid ($PGF_{2\alpha}$)-contracted pulmonary artery were increased, compared with those of controls (Wanstall et al., 1993). In contrast, chronic hypoxia-induced pulmonary hypertension decreased sensitivity to sodium nitroprusside (Wanstall and O'Donnell, 1992; Crawley et al., 1992). In our study, there was no apparent difference between the sodium nitroprusside-, pinacidil- and nicorandil-induced relaxation of the pre-contracted aorta and pulmonary artery of both strains. The involvement of activation of guanylate cyclase in the sodium nitroprusside-mediated vascular muscle relaxation was confirmed by the inhibition of sodium nitroprusside

responses in the presence of LY 83583, a soluble guanylate cyclase inhibitor (Fig. 3). Therefore, the results of the present study are consistent with those of studies by Shirasaki et al. (1986) and Lograno et al. (1989), in which sodium nitroprusside- and cGMP-mediated relaxation was not 'affected' by the hypertensive state of the animal examined.

It has been reported that nicorandil, an ATP-sensitive K^+ channel opener, produces smooth muscle relaxation by multiple mechanisms. In addition to the well-known effects on the opening of ATP-sensitive K^+ channels, nicorandil-induced relaxation also involves the activation of guanylate cyclase as well as the opening of Ca^{2+}-activated K^+ channels (Zhou et al., 1995). Similar to the results for sodium nitroprusside, pre-incubation of the tissues with LY 83583 (1 μM) abolished the nicorandil-mediated relaxation observed in the aorta and pulmonary artery of both strains (Fig. 3). However, no apparent change in the nicorandil concentration–response curve was observed in the presence of glibenclamide (3 μM) (Fig. 2), charybdotoxin (100 nM) or apamin (1 μM). These results suggested that the nicorandil-induced relaxation solely involved the activation of guanylate cyclase. The underlying mechanism responsible for the 'unaltered' relaxation responses to pinacidil in SHR tissue preparations remains to be determined.

KR-30450 is a recently synthesized benzopyran derivative which possesses ATP-sensitive K^+ channel opener activities (Kwak et al., 1995) with an IC_{50} of 3 μM in rat papillary muscle. The effect of KR-30450 on action potential duration in rat papillary muscle (Kwak et al., 1995) could be reversed by glibenclamide, a specific ATP-dependent K^+ channel blocker (Belles et al., 1987; Hamada et al., 1990). In our study, the IC_{50} values determined for the vascular smooth muscle preparations (isolated aorta and pulmonary artery of both species) were in the range of 60–80 nM (Tables 1 and 2). This tissue difference was also seen with other K^+ channel openers and not only with KR-30450. Besides, 3 μM glibenclamide caused a parallel rightward shift of the concentration–response curve for KR-30450 in WKY rat aorta (Fig. 2), as well as in the WKY pulmonary artery, and SHR aorta and pulmonary artery. However, charybdotoxin (100 nM) and apamin (1 μM) failed to modify the KR-30450-induced relaxation responses in aorta and pulmonary artery from both strains. These results suggested that KR-30450 activates ATP-dependent K^+ channels and causes relaxation.

It has been reported that nicorandil, an ATP-sensitive K^+ channel opener, has a $-NO_2$ group in its chemical structure (Cook, 1988; Edwards and Weston, 1990). In addition to the opening of K^+ channels, it has been demonstrated that nicorandil can activate guanylate cyclase (thereby increasing the intracellular cGMP level) in vascular smooth muscle and hence, cause vasorelaxation (Kukovetz et al., 1991; Zhou et al., 1995). It is interesting to note that there is also a $-NO_2$ group in the chemical structure of KR-30450 (Kwak et al., 1995). The presence of this $-NO_2$ group, however, did not enable this compound to act as an activator of guanylate cyclase in either pulmonary artery or aorta in this study. This conclusion was reached because, unlike in the experiments with nicorandil and sodium nitroprusside, LY 83583 (1 μM, a soluble guanylate cyclase inhibitor) failed to modify the KR-30450-mediated relaxation responses. Perhaps the position of the $-NO_2$ group in the chemical structure is important in determining the ability of a particular drug to activate guanylate cyclase, but further studies are required to clarify the exact mechanism(s).

Aprikalim has been shown to relax rat isolated aorta (Greenwood and Weston, 1993) and rabbit isolated aorta and pulmonary artery, an effect which was inhibited by glibenclamide (Magnon et al., 1994). In this study, the vasorelaxant effect of aprikalim was also attenuated in the presence of 3 μM glibenclamide and a rightward shift of the concentration–response curve of aprikalim was recorded. Similar to the results observed in rabbit isolated aorta and pulmonary artery preparations (Magnon et al., 1994), activation of guanylate cyclase was not involved since the aprikalim-mediated responses were not affected by LY 83583 (Fig. 3). In addition, pre-incubation of the tissue preparations with charybdotoxin (100 nM) and apamin (1 μM) failed to affect the vasorelaxant effect of aprikalim. These results indicated that the aprikalim-induced vascular relaxant response in the aorta and pulmonary artery of both strains solely involves the activation of ATP-sensitive K^+ channels.

In the present studies, administration of cromakalim caused a concentration-dependent relaxation of the precontracted aorta and pulmonary artery from both strains. The vasorelaxant response to cromakalim was significantly attenuated by glibenclamide (3 μM) and a rightward shift of the concentration–response curve of cromakalim was observed (Fig. 2). In contrast to other preparations e.g., rabbit aorta (Gelband et al., 1989), rat portal vein (Hu et al., 1990) and rat basilar artery (Stockbridge et al., 1991), the presence of charybdotoxin and apamin failed to modify the relaxation elicited by cromakalim in aorta and pulmonary artery. The involvement of guanylate cyclase can also be ruled out (Satake et al., 1995) because the presence of LY 83583 did not affect the cromakalim-induced relaxation (Fig. 3).

In general, the pulmonary relaxant effects of ATP-sensitive K^+ channel openers, except pinacidil in fetal lambs (Chang et al., 1992), are not dependent on the presence of the endothelium (O'Donnell et al., 1991; Savineau and Marthan, 1993). The failure of L-NAME (20 μM), a NO synthase inhibitor, to affect the vasorelaxant effects of sodium nitroprusside and all ATP-dependent K^+ channel openers examined support the idea that the release of NO did not play a role in the observed vasorelaxation produced by these compounds.

In treating pulmonary hypertension, the single most

important problem has been the absence of drugs that act selectively on the pulmonary artery with little or no effect on the systemic circulation. However, the drugs examined in this study (sodium nitroprusside and all ATP-sensitive K^+ channel openers) did not have a selective relaxant effect in the pulmonary artery relative to the thoracic aorta in both SHR and WKY rats. Our results also showed that the SHR pulmonary artery preparation, unfortunately, does not seem to offer any advantage compared to the normotensive WKY pulmonary artery preparation when examining the possible anti-hypertensive effects of ATP-dependent K^+ channel openers or sodium nitroprusside. The drugs were equipotent in relaxing thoracic aorta and pulmonary artery from both strains, except KR-30450, aprikalim and cromakalim, which were more potent in relaxing SHR thoracic aorta at higher concentrations. Nevertheless, this study demonstrated that ATP-dependent K^+ channel openers showed different relaxant properties in thoracic aorta and pulmonary artery isolated from SHR and WKY rats. The vascular relaxant effect of KR-30450, aprikalim, cromakalim and pinacidil involved the opening of ATP-sensitive K^+ channels whereas the responses elicited by nicorandil and sodium nitroprusside were due to the activation of soluble guanylate cyclase. Among all the drugs tested, sodium nitroprusside was the most potent and nicorandil was the least potent.

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