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Differential Vasorelaxant Effects of KR-30075, a New Cyclic AMP-phosphodiesterase Inhibitor, on Guinea-pig Pulmonary, Bovine Coronary and Renal Arteries

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Abstract□The vasorelaxant effects of KR-30075 in guinea-pig pulmonary, bovine coronary and renal arterial strips contracted by either K^+-depolarization, phenylephrine, or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were evaluated. KR-30075 was more potent than imazodan as a vasorelaxant against $PGF_{2\alpha}$-induced contractions in bovine coronary and renal arteries, whereas against K^+-induced contractions KR-30075 was less potent than imazodan in guinea-pig pulmonary arteries and more potent in bovine coronary arteries. KR-30075 was more potent against contractions induced by phenylephrine or $PGF_{2\alpha}$ than the contractions induced by K^+. This profile of activity for KR-30075 was similar to that of imazodan and dissimilar from the calcium entry blocking agent nifedipine. There was no vascular selectivity of KR-30075 between bovine coronary and renal arterial strip preparations. In conclusion, this study shows that KR-30075 represents the vasorelaxant effects on guinea-pig pulmonary, bovine coronary and renal arteries without specific vascular selectivity. The vasorelaxant profile of KR-30075, with different sources of vascular smooth muscle, is unlike that of calcium entry blocking agent and more similar to the profile of the agent that inhibit cyclic nucleotide phosphodiesterase.

Key words□KR-30075, imazodan, PDE inhibitor, vasorelaxant effect, vascular smooth muscle

Pharmacotherapy of congestive heart failure is based on two principles; unloading of the heart and inotropic stimulation[1]. Unloading is achieved through vasodilation with afterload and/or preload reduction, or through diuresis with reduction of cardiac filling and improvement of vascular compliance. Unloading of the heart results in reduced myocardial oxygen consumption and improves the working conditions of the contractile elements. Inotropic stimulation by itself requires energy, i.e., oxygen. The increase in oxygen consumption may exceed the improvement in force reduction. This oxygen-wasting effect has been demonstrated in the working papillary muscle for β-stimulating agents as well as for phosphodiesterase inhibitors. In the clinical situation, concomitant vasodilation of a given inotropic agent may conceal the oxygen demand of inotropism. For this very reason the combination of inotropic stimulation and vasodilation can be favorable[2-4]. Newly developed cAMP-phosphodiesterase (PDE) inhibitors such as milrinone, fenoximone and imazodan have been reported to represent both positive inotropic and vasodilation effect[5-11].

KR-30075 (4, 5-dihydro-6-[3-nitro-4-methoxyphenyl]-5-methyl-3 [2H]-pyridazinone) is a newly synthesized pyridazinone compound. As the previous reports have shown, this agent represented a selective inhibitory effect on cAMP-PDE III isoenzyme and produced a positive inotropic effect in the isolated guinea-pig atria and Langendorff heart preparation[12, 13]. Previous study in this laboratory has suggested the possibility of vasorelaxant effect of KR-30075 for the reason it increased the coronary flow rate in guinea-pig Langendorff heart and decreased the blood pressure in spontaneously hypertensive rat (SHR)[14].

The purpose of this study was to evaluate the direct effects of KR-30075 on several smooth muscle preparations and to compare its activity with that of other known vasorelaxants. This study included an evaluation of the mode of action of the vasorelaxant effect of KR-30075 by comparing the effects in voltage-dependent (K^+-depolarization) versus agonist-induced (receptor operated) contractions of guinea-pig pulmonary arterial strips[15]. These studies were accomplished by contracting the blood vessels with either a high K^+ or a α-agonist phenylephrine. Isolated coronary and renal arterial rings or strips are commonly used by vascular smooth muscle investiga-

tors. We also tried to investigate the profile of KR-30075 in different isolated arterial preparations. The differential effects of KR-30075 were evaluated in bovine coronary and renal arterial strips contracted by K^+ or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In addition, the vascular selectivity of KR-30075 for those two tissues was evaluated. For comparison, imazodan (cAMP-PDE III inhibitor) and nifedipine (dihydropyridine calcium entry blocker) were used in these studies.

METHODS AND MATERIALS

Guinea-pig pulmonary artery preparations

Male guinea-pigs (300-350g) were killed by a blow to the head and the pulmonary artery was rapidly excised. The tissue was cleaned of connective tissue and cut helically into strips of about 20 to 25 mm in length, 1.5 mm in width. Contractions were measured by mounting the arterial strips with two stainless-steel clips, one of which was connected to a lever transducer (HSE D7801). The strips were immersed in a 20 ml organ bath filled with Tyrode solution of the following composition (mM): NaCl, 112; KCl, 5; $CaCl_2$, 1.2; $MgSO_4$, 0.56; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 12. A gas mixture of 95% O_2-5% CO_2 was left to equilibrate under 1g of resting tension for 1 hr before beginning of the experiment. Isotonic change was measured and recorded on Multicorder (HSE MC6625).

Bovine coronary and renal artery preparations

Bovine hearts and kidneys were obtained from a local slaughterhouse, and kept in an ice-cold Tyrode solution during the transportation. Left circumflex arteries and renal arteries were roughly dissected and taken to the laboratory within 2 hr after the animals were sacrificed. After removing all fat and connective tissue, the arteries were everted, cut into spiral strips measuring approximately 2-3 mm in width and 15 mm in length. The strips were vertically mounted in 20 ml organ baths filled with Tyrode solution, maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Changes in force were recorded isometrically using a Grass model 7E polygraph connected to a Grass FT 03 force displacement transducer. The vessel preparations were allowed to equilibrate for 2-3 hr under passive forces of 1.5g (readjusting applied force to 1.5g as needed during the equilibration period), after which the contractile response of each tissue was tested twice by addition of 60 mM KCl. Any strip not attaining 0.5g of active force was eliminated from further study at this point. After washing, the tissues were equilibrated for an additional 30 min before each experiment.

Vasorelaxant effects of compounds on guinea-pig or bovine arterial strips

In initial experiments, the concentration-response relation to K^+, phenylephrine and $PGF_{2\alpha}$ was studied to determine the concentrations of these agents that gave approximately 80% of the maximal response. These concentrations (60 mM KCl, 10 μM phenylephrine and 20 μM $PGF_{2\alpha}$) were subsequently used to elicit contractions that were sustained over a 1-3 hr period. After stable contractile responses were obtained to the selected agonists (K^+ or phenylephrine or $PGF_{2\alpha}$), the vasorelaxant effects of the compound under investigation were determined after the contractions had reached plateau (approximately 10-30 min) by addition of cumulatively increasing concentrations to the bath. Changes in force were recorded in grams and reported as percent relaxation of maximal contraction. Activity of the relaxant was calculated by following the probit method[16-18] and expressed as EC_{50} (concentration of vasorelaxant that causes a 50% relaxation of maximum contraction). The log transformed EC_{50} values were compared by student's T-test. The level of significance was assigned at $p<0.05$. The EC_{50} values were presented as geometric means with their associated 95% confidence intervals.

Drugs and chemicals used in this study were prostaglandin $F_{2\alpha}$, phenylephrine, nifedipine (Sigma Chemical Co.), imazodan and KR-30075 (Korea Research Institute of Chemical Technology).

RESULTS

Guinea-pig pulmonary arteries

Fig. 1 displays the effects of KR-30075, imazodan and nifedipine on guinea-pig pulmonary arterial strips constricted by either K^+ (60 mM) or phenylephrine (10 μM). KR-30075 and imazodan were more potent against contractions induced by phenylephrine than contractions induced by K^+, but nifedipine was more potent against K^+-induced contractions than phenylephrine contractions. KR-30075 and imazodan caused relaxation at concentrations ranging from 10^{-8} to 10^{-5} M, whereas nifedipine began to produce relaxation at concentrations less than 10^{-9} M. Maximal relaxation of K^+-contracted vessels was not reached because of limitation in the solubility of KR-30075 and imazodan. The difference in the slopes of the concentration-relaxation curves between K^+-contraction and phenylephrine-contraction was shown for KR-30075. In guinea-pig pulmonary arterial strips contracted by KCl, the EC_{50} for KR-30075 was significantly ($p<0.05$) higher than those for imazodan and nifedipine (Table I).

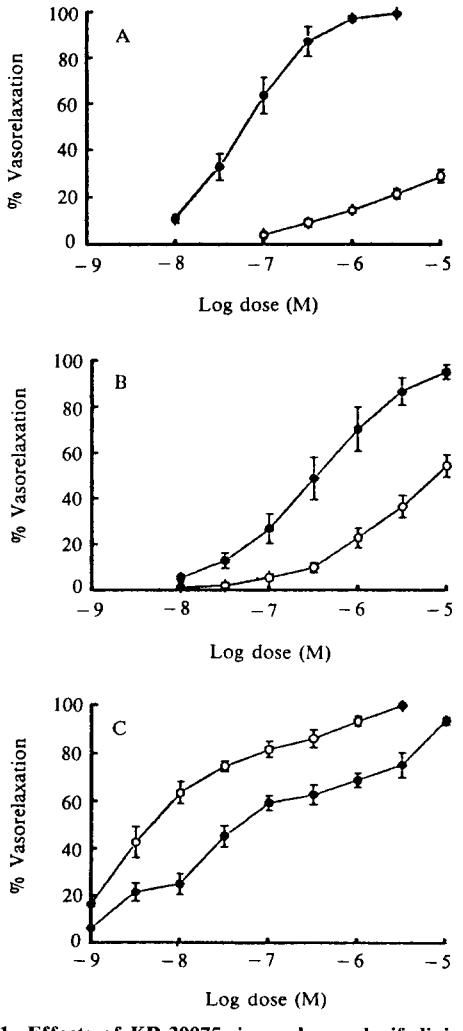

Fig. 1. Effects of KR-30075, imazodan and nifedipine in K^+ or phenylephrine contracted guinea-pig pulmonary artery.

Cumulative concentration-relaxation relations for each compound against phenylephrine (-●-) or K^+ (-○-) contracted pulmonary arterial strips from guinea-pig are shown. Each point represents the mean ± SEM for n = 4-6. A: KR-30075, B: Imazodan, C: Nifedipine

Bovine coronary arteries

KR-30075, imazodan and nifedipine caused concentration-dependent relaxation of bovine coronary arteries contracted by either K^+ or $PGF_{2\alpha}$ (Fig. 2). As also shown in Fig. 2, KR-30075 and imazodan were more potent in arteries contracted with $PGF_{2\alpha}$ than in arteries contracted with K^+, however, nifedipine showed opposite profile of vasorelaxant effect against different constrictors, K^+ or $PGF_{2\alpha}$. Against K^+- or $PGF_{2\alpha}$-induced contractions, the EC_{50} for KR-30075 was significantly ($p<0.01$) lower than the EC_{50} for imazodan (Table II).

Table I. Effects of KR-30075, imazodan and nifedipine on contractile responses to KCl and phenylephrine in guinea-pig pulmonary arterial strips

	EC_{50} (95% Confidence Intervals) (μM)[a]			
	KCl (60 mM)	n	Phenylephrine (10 μM)	n
KR-30075	72.6 (26.9-196)[c]	4	0.058 (0.029-0.116)[b]	5
Imazodan	7.39 (3.11-17.6)	5	0.322 (0.089-1.17)[b]	5
Nifedipine	0.005 (0.002-0.014)	6	0.101 (0.039-0.261)[b]	5

EC_{50}: concentration giving 50% relaxation.

[a] Concentration curves for relaxation were determined for each vasodilator and the EC_{50} was determined by probit analysis. Geometric means and 95% confidence limits were determined.

[b] EC_{50} value is significantly different ($p<0.01$) from that for KCl-contracted arteries.

[c] EC_{50} value is significantly different ($p<0.05$) from that for other agents.

Bovine renal arteries

As shown in Fig. 3, bovine renal arterial strips contracted by either K^+ or $PGF_{2\alpha}$ were relaxed by exposure to KR-30075, imazodan and nifedipine at concentrations ranging from 10^{-8} to 10^{-5} M. KR-30075 and imazodan were more potent against contractions induced by $PGF_{2\alpha}$ than by contractions induced by 60 mM K^+. Nifedipine was equipotent regardless of the different constrictors. In renal arterial strips contracted by $PGF_{2\alpha}$ (20 μM) the EC_{50} for KR-30075 was significantly lower than that for imazodan ($p<0.05$) (Table II).

As also shown in Table II in which EC_{50} for three compounds are compared, KR-30075 and imazodan were significantly more potent ($p<0.01$) against contractions induced by $PGF_{2\alpha}$ than contractions induced by K^+ in both coronary and renal arteries, while infedipine was significantly ($p<0.01$) more potent vasorelaxant against K^+-induced contractions than $PGF_{2\alpha}$-induced contractions in coronary artery.

DISCUSSION

This study demonstrates that KR-30075 relaxes K^+- or phenylephrine-contracted guinea-pig pulmon-

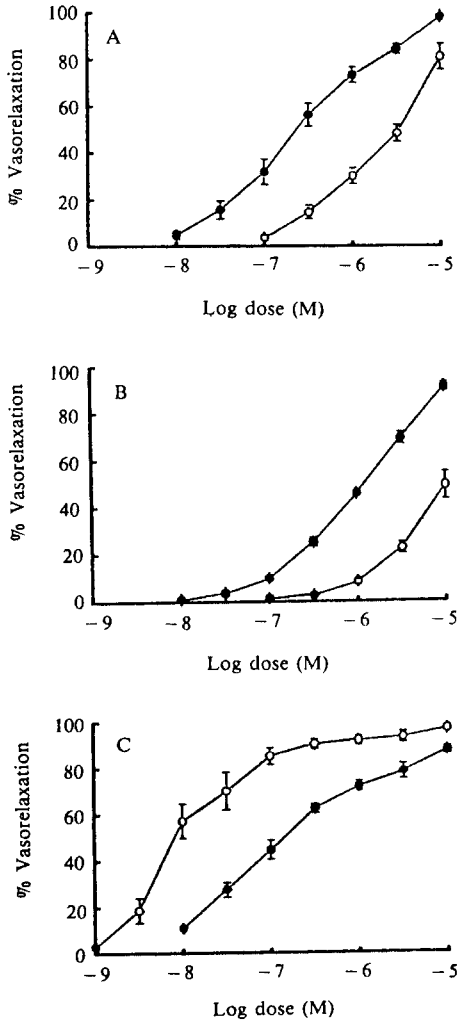

Fig. 2. Effects of KR-30075, imazodan and nifedipine in K^+ or $PGF_{2\alpha}$ contracted bovine coronary artery. Cumulative concentration-relaxation relations for each compound against $PGF_{2\alpha}$ (-●-) or K^+ (-○-) contracted arterial strips from bovine coronary are shown. Each point represents the mean ± SEM for n = 3-5. A: KR-30075, B: Imazodan, C: Nifedipine

Table II. Comparison of the relaxant effects of KR-30075, imazodan and nifedipine on K^+ or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) contracted bovine coronary or renal arterial strip preparations

	EC_{50} (95% Confidence Intervals) (μM)[a]			
	Coronary	n	Renal	n
KR-30075				
K^+	2.56 (1.35-4.87)[c]	5	52.7 (8.47-328)	4
$PGF_{2\alpha}$	0.259 (0.128-0.521)[bc]	5	0.172 (0.052-0.568)[bc]	3
Imazodan				
K^+	13.3 (4.99-35.6)	5	64.9 (17.5-240)	3
$PGF_{2\alpha}$	1.18 (1.06-1.32)[b]	5	0.613 (0.263-1.43)[b]	4
Nifedipine				
K^+	0.012 (0.006-0.021)	4	0.106 (0.047-0.238)	4
$PGF_{2\alpha}$	0.201 (0.116-0.347)[b]	5	0.106 (0.053-0.209)	4

EC_{50}: concentration giving 50% relaxation.

[a] Concentration curves for relaxation were determined for each vasodilator and the EC_{50} values were determined by probit analysis. Means were calculated as geometric means with 95% confidence intervals.

[b] EC_{50} value is significantly different ($p<0.01$) from that for other contractile agent in the same artery.

[c] EC_{50} value is significantly different ($p<0.05$) from that for imazodan in the same artery contracted by the same agent.

ary arterial strips and K^+- or $PGF_{2\alpha}$-contracted bovine coronary, renal arterial strips. These results are consistent with the previous Langendorff heart studies which demonstrated an increased coronary flow rate by KR-30075, by showing the KR-30075 is a direct coronary vasodilator[14]. KR-30075 is more potent than imazodan as a vasorelaxant against $PGF_{2\alpha}$-induced contractions in bovine coronary and renal arteries, while against K^+-induced contractions KR-30075 is less potent than imazodan in guinea-pig pulmonary arteries and more potent than imazodan in bovine coronary arteries.

This study also investigated the profile of vasorelaxant activity of KR-30075 against different vasoconstrictors in guinea-pig pulmonary artery. The vasorelaxant mechanism of action of KR-30075 is distinctly different from that of the voltage-dependent calcium entry blocking agents. Calcium entry blockers, such as nifedipine, are more potent vasorelaxants against contractions induced by K^+-depolarization (Voltage-dependent contractions) than from those induced by receptor mediated mechanisms[19, 20]. The opposite profile was apparent with KR-30075 because it was significantly more potent against contractions initiated by receptor-mediated (phenylephrine) mechanisms than against those induced by K^+-depolarization. These results suggest that KR-30075 does not produce vasorelaxation by way of a voltage-sensitive calcium channel blockade. One possible mechanism of action

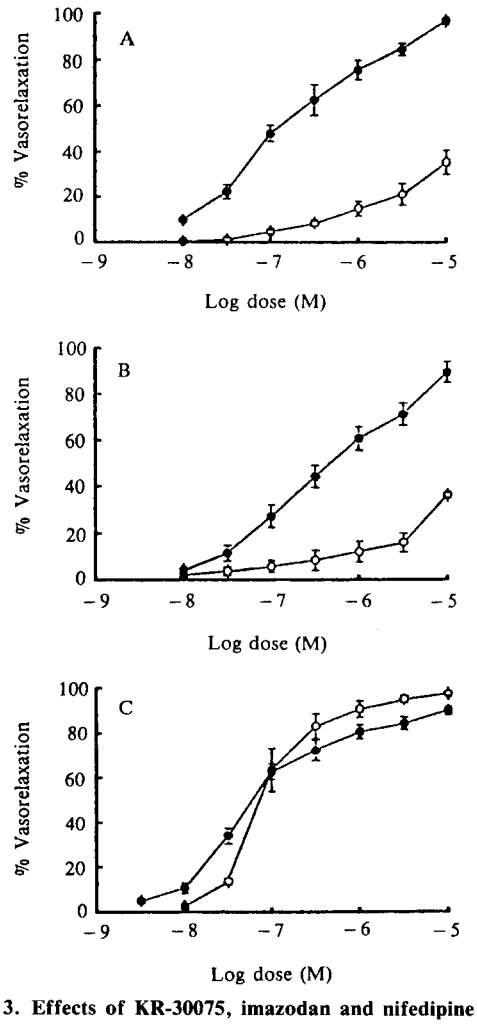

Fig. 3. Effects of KR-30075, imazodan and nifedipine in K^+ or $PGF_{2\alpha}$ contracted bovine renal artery. Cumulative concentration-relaxation relations for each compound against $PGF_{2\alpha}$ (-●-) or K^+ (-○-) contracted renal arterial strips from bovine are shown. Each point represents the mean ± SEM for n = 3-5. A: KR-30075, B: Imazodan, C: Nifedipine

that may explain this vasorelaxant profile for KR-30075 may be related to increase in cyclic nucleotides. KR-30075 has been shown to be a selective inhibitor of cAMP-PDE III isozyme[12, 13]. Vasorelaxant profile of KR-30075 was consistently similar to that of imazodan which is a pyridazinone derivative PDE III inhibitor together with vasodilator action[21-23].

In the further studies with bovine coronary and renal arterial strips, KR-30075 and imazodan were more potent in arteries contracted with $PGF_{2\alpha}$ than in arteries contracted with K^+. Nifedipine, however, showed opposite profile of vasorelaxant effect against different constrictors in bovine coronary artery. The slopes of the concentration-relaxation curves for all three compounds are similar in coronary arteries contracted by either agent. In coronary arteries contracted by either K^+ or $PGF_{2\alpha}$, KR-30075 is more potent vasorelaxant than imazodan. There is not significant vascular selectivity of KR-30075 for bovine coronary and renal arterial strip preparations.

The bovine renal artery, in this study, was equisensitive to nifedipine during K^+ or $PGF_{2\alpha}$-induced contractions. This would suggest that $PGF_{2\alpha}$-mediated contraction in bovine renal arteries may arise primarily from calcium entry through voltage sensitive calcium channels. This is supported by the previous reports which showed the equisensitivity of dog renal artery to calcium entry blocker, nimodipine, during K^+- or agonist-induced contraction[24]. Moreover, Harris *et al.* demonstrated the lack of efficacy of the receptor-mediated vasorelaxant isoproterenol in both K^+ and agonist-induced contractions of dog renal arteries[24]. Interestingly, the mode of action of KR-30075 that produced more potent vasorelaxant effect in $PGF_{2\alpha}$-contraction than K^+-contraction is similar in either coronary or renal arteries. This suggests the possibility that one or more additional subcellular mechanisms may be included for the vasorelaxant action of KR-30075 in renal artery.

In conclusion, the present study has demonstrated that the vasorelaxant profile of KR-30075 in guinea-pig pulmonary, bovine coronary and renal arteries is unlike the voltage-dependent calcium entry blocker, but is similar to that of imazodan which is cAMP-PDE III inhibitor. This study has also shown that KR-30075 does not represent vascular selectivity for coronary or renal arteries. The data obtained in these and other previous studies suggest the possibility of KR-30075 to be an effective positive inotropic and vasodilator substance *in vivo*. Further studies on dog hemodynamic activity and toxic effect of this compound are being performed.

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