J. Pharm. Pharmacol. 1993, 45: 573–575
Communicated June 24, 1992

Comparison of the effects of KRN2391 and other coronary dilators on porcine isolated coronary arteries of different sizes

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Abstract—The present study was performed to determine whether KRN2391 (*N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate), a novel vasodilator, shows different effects on porcine isolated coronary arteries of different sizes. The vasodilating effects of KRN2391 on porcine large (2·5–3·0 mm outer diam.) and small (0·8–1·0 mm) coronary arteries were also compared with those of cromakalim, nicorandil, nifedipine, nitroglycerin and adenosine. The relaxant effects of these drugs were examined in coronary arteries contracted by 25 mM KCl. Nitroglycerin caused greater relaxation in large vessels than in small vessels. In contrast, adenosine, nifedipine and cromakalim caused greater relaxation in small vessels. However, there was no difference between large and small vessels in the relaxing effects of KRN2391 and nicorandil. These unique features of KRN2391 and nicorandil appear to be beneficial in ischaemic heart disease.

Nitroglycerin and calcium-channel blockers are prescribed clinically for angina pectoris. The beneficial effects of these drugs are thought to derive from reduction of preload or afterload on the heart, and to increase oxygen supply based on an increase in coronary blood flow (Godfraind et al 1986; Taira 1987). The redistribution of coronary blood flow from normal areas to ischaemic areas is an important factor in treatment of the ischaemic heart (Winbury et al 1969). It has been known for many years that dilation of the large epicardial coronary artery, rather than coronary resistance arterioles, contributes to the redistribution of coronary blood flow. Coronary dilators which are highly selective for small resistance vessels may cause inappropriate distribution of blood flow stemming from an excess in areas where it is not required at the expense of ischaemic areas (Winbury et al 1969).

Recently, we synthesized KRN2391, *N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate, which has a potent vasorelaxant effect through a potassium-channel opening action and a nitrate-like action (Kashiwabara et al 1991). In in-vivo experiments using anaesthetized dogs, KRN2391 showed a marked and selective increase in coronary blood flow (Ogawa et al 1990). Therefore, KRN2391 is expected to be useful for ischaemic heart disease. In the present study, we compared the relaxant effect of KRN2391 on coronary arteries of different sizes with nitroglycerin, nicorandil and nifedipine used as antianginal drugs. The relaxant effects of adenosine and cromakalim, a potassium-channel opener, were also examined.

Materials and methods

Fresh porcine hearts were obtained from a local abattoir and immediately immersed in ice-cold Krebs–Henseleit solution of the following composition (mM): NaCl 118·0, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25·0, glucose 10.0.

The anterior descending branch (epicardial location, 2·5–3·0 mm outer diam.) and transmural location (0·8–1·0 mm outer diam.) of the left coronary arteries were isolated as a large and a small size, respectively. The arteries were cut into 2 mm rings. The rings were then mounted between two stainless steel triangular hooks and suspended in a 10 mL organ bath filled with Krebs–Henseleit solution which was maintained at 37°C and gassed with 95% O_2–5% CO_2. The tension of each segment was measured isometrically with a force-displacement transducer (Nihon Kohden, TB-611T). Tension of the strips was adjusted to 19·6 mN on the large vessel and 4·9 mN on the small vessel, which produced the maximal contraction obtained by KCl. After an equilibration period of 120 min, the preparations were contracted by changing the solution in the bath to one containing 25 mM KCl. This potassium-depolarizing solution was prepared by replacing 20·3 mM NaCl with 20·3 mM KCl in the control solution. After the arteries reached a stable tension, vasodilators were administered in a cumulative manner.

Drugs. KRN2391, nicorandil and cromakalim were synthesized in our laboratory. Adenosine and nifedipine were purchased from Sigma (USA). Nitroglycerin was obtained from Nippon Kayaku Co. (Japan). Nicorandil, cromakalim and nifedipine were dissolved in 0·1 M HCl, dimethylsulphoxide and ethanol, respectively. All other drugs were dissolved in double-distilled water. These stock solutions were diluted in Krebs–Henseleit solution when necessary. In preliminary experiments, we confirmed that the vehicle for each test drug had no effect on small and large coronary arteries contracted by 25 mM KCl.

Statistical analysis. Relaxation caused by the test drugs was expressed as a percentage of the maximum relaxation obtained at the end of each experiment by addition of 10^{-4} M papaverine. All results are presented as mean ± s.e.m. The mean EC50 or EC30 value was determined from the concentration–relaxation curves which were fitted by linear regression. Difference was evaluated at a significance of $P < 0.05$ using a *t*-test.

Results

KRN2391 (10^{-8}–3×10^{-5} M), nitroglycerin (10^{-9}–10^{-5} M), nicorandil (10^{-8}–3×10^{-4} M), adenosine (10^{-6}–3×10^{-4} M), cromakalim (10^{-8}–3×10^{-5} M) and nifedipine (10^{-12}–10^{-7} M) produced concentration-dependent relaxation in large and small coronary arteries contracted by 25 mM KCl (Figs 1, 2).

In large coronary arteries, the potency (EC50) on contraction induced by 25 mM KCl was as follows: nifedipine > KRN2391 > nitroglycerin > cromakalim > nicorandil > adenosine, and in small coronary arteries: nifedipine > KRN2391 > cromakalim > nitroglycerine > nicorandil > adenosine. The relaxation induced by KRN2391 and nicorandil was nearly 100% at their maximum effective doses in large and small coronary arteries. The maximum relaxation with nitroglycerin, adenosine, cromakalim and nifedipine could not be examined because test solutions of high concentration could not be made. However, relaxation induced by cromakalim reached the plateau at more than 3×10^{-6} M; the relaxation at 3×10^{-5} M cromakalim was about 69·0% (n = 5) and 87·5% (n = 5) in large and small coronary arteries, respectively.

The relaxant effect of nitroglycerin in large coronary arteries was observed from a lower dose compared with that in small arteries. In contrast, the relaxant effects of adenosine, cromaka-

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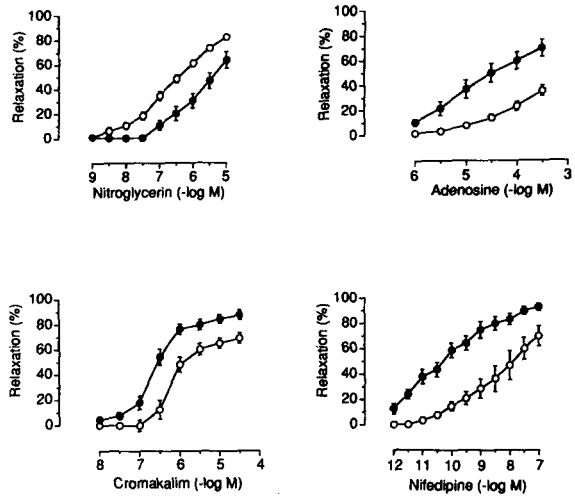

FIG. 1. Concentration–relaxation curves for nitroglycerin, adenosine, cromakalim and nifedipine in porcine large (○) and small (●) coronary arteries contracted by 25 mM KCl. Each point represents the mean ± s.e.m. (n = 4–7).

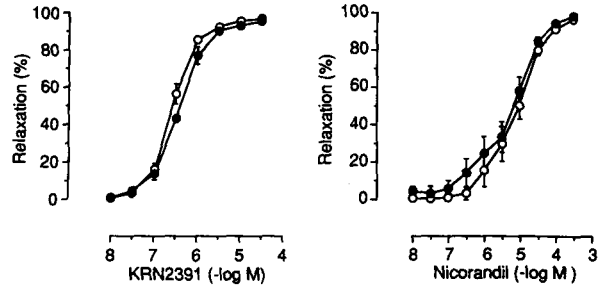

FIG. 2. Concentration–relaxation curves for KRN2391 and nicorandil in porcine large (○) and small (●) coronary arteries contracted by 25 mM KCl. Each point represents the mean ± s.e.m. (n = 5–6).

lim and nifedipine were observed from a lower dose in small coronary arteries rather than in large vessels. The concentration–response curves for KRN2391 and nicorandil in large and small coronary arteries overlapped.

The EC50 or EC30 values are shown in Table 1. The EC50 value of nitroglycerin was greater in small rather than large coronary arteries. The EC50 values of cromakalim and nifedipine and the EC30 value of adenosine were greater in large coronary arteries than in small vessels. There was no significant difference between large and small coronary arteries in the EC50 values of KRN2391 and nicorandil.

Discussion

In the present study, the different profiles among coronary dilators were observed in relaxing large and small coronary arteries. Such a difference in effect between large and small coronary arteries is thought to influence the redistribution of coronary blood flow and an increase in coronary blood flow (Ishibashi et al 1989); that is, dilation of large coronary arteries is associated with the redistribution of coronary blood flow supply to ischaemic areas, and that of small coronary arteries to the inappropriate distribution of coronary blood flow (Winbury et al 1969; Schwartz et al 1979).

The potent relaxing effect of nitroglycerin on large coronary arteries is in agreement with the results of Schnaar & Sparks (1972) and Sellke et al (1990). Sellke et al (1990) suggested that this effect with nitroglycerin may be caused by a relative deficiency of available sulphydryl groups or a lack of enzyme necessary for conversion of nitroglycerin to its active metabolites in small coronary resistance vessels. Thus, it is considered that nitroglycerin shows the beneficial effect of redistribution of coronary blood flow rather than an increase in coronary blood flow. Indeed, it has been reported that the effect of nitroglycerin in increasing coronary blood flow is less potent than those of adenosine, nifedipine and cromakalim, which show potent relaxation of small coronary arteries in-vivo (Millard et al 1983; Sakanashi et al 1986; Kato et al 1987; Giudicelli et al 1990). Furthermore, nitroglycerin hardly produces dilation of small coronary arteries in conscious dogs (Giudicelli et al 1990).

The present results also show the potent relaxing effects of adenosine, cromakalim and nifedipine in small coronary arteries. These results for adenosine and nifedipine are supported by reports from Schnaar & Sparks (1972) and Takeda et al (1977). Thus, these drugs are thought to increase coronary blood flow, and the intravenous administration of adenosine, cromakalim or nifedipine produced a potent increase in coronary blood flow in anaesthetized dogs (Millard et al 1983; Kato et al 1987; Giudicelli et al 1990). However, these drugs may induce a disadvantageous effect on the redistribution of coronary blood flow because they show less potent relaxation of large coronary arteries which play an important role in redistribution of blood flow in the heart. Indeed, it has been reported that dipyridamole,

Table 1. The EC50 values and the maximum relaxation caused by KRN2391, nicorandil, nitroglycerin, cromakalim, nifedipine and adenosine in porcine large and small coronary arteries.

	Large coronary arteries		Small coronary arteries	
Vasodilator	EC50 (M)	Maximum relaxation (%)	EC50 (M)	Maximum relaxation (%)
KRN2391	$(3{\cdot}58 \pm 0{\cdot}58) \times 10^{-7}$	$96{\cdot}8 \pm 0{\cdot}6$	$(4{\cdot}78 \pm 0{\cdot}68) \times 10^{-7}$	$95{\cdot}3 \pm 1{\cdot}0$
Nicorandil	$(9{\cdot}31 \pm 2{\cdot}58) \times 10^{-6}$	$96{\cdot}7 \pm 0{\cdot}6$	$(6{\cdot}02 \pm 2{\cdot}58) \times 10^{-6}$	$98{\cdot}4 \pm 0{\cdot}7$
Nitroglycerin	$(5{\cdot}29 \pm 1{\cdot}38) \times 10^{-7}$	$81{\cdot}3 \pm 1{\cdot}7$	$(5{\cdot}92 \pm 2{\cdot}50) \times 10^{-6}$*	$63{\cdot}1 \pm 6{\cdot}7$*
Cromakalim	$(3{\cdot}19 \pm 1{\cdot}33) \times 10^{-6}$	$69{\cdot}0 \pm 4{\cdot}2$	$(7{\cdot}03 \pm 1{\cdot}19) \times 10^{-7}$*	$87{\cdot}5 \pm 3{\cdot}9$**
Nifedipine	$(3{\cdot}18 \pm 1{\cdot}73) \times 10^{-8}$	$69{\cdot}8 \pm 8{\cdot}0$	$(1{\cdot}72 \pm 1{\cdot}33) \times 10^{-10}$*	$92{\cdot}5 \pm 3{\cdot}0$*
Adenosine	$(2{\cdot}75 \pm 1{\cdot}21) \times 10^{-4}$ (EC30)	$36{\cdot}0 \pm 4{\cdot}2$	$(2{\cdot}43 \pm 1{\cdot}81) \times 10^{-5}$* (EC30)	$70{\cdot}3 \pm 6{\cdot}7$**

EC50 values and maximum relaxation of concentration–relaxation curves for KRN2391, nicorandil, nitroglycerin, cromakalim, nifedipine, and adenosine in porcine coronary arteries pre-contracted with 25 mM KCl. With adenosine, the EC30 was used because relaxation of more than 50% in large coronary arteries did not occur. Values represent the mean ± s.e.m. (n = 4 ~ 7). *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ vs large coronary arteries.

which potentiates the effect of adenosine, and nifedipine induce coronary steal (Wilcken et al 1971; Yokoyama et al 1982).

The relaxation effects of KRN2391 and nicorandil were equipotent in both large and small coronary arteries. They have been shown to possess the dual vasodilatory mechanism of a nitrate and a potassium channel-opener (Taira 1989; Kashiwabara et al 1991). In particular, in the coronary circulation of dogs, KRN2391 and nicorandil behave predominantly as a nitrate in large coronary arteries and as a potassium-channel opener in resistive coronary arterioles (Yoneyama et al 1990; Satoh et al 1991; Fukata et al 1991; Kingsbury et al 1991). Recently, we also observed that KRN2391 and nicorandil increased cGMP formation in porcine large coronary arteries (Jinno et al 1992). Thus, such a dual action of KRN2391 and nicorandil is thought to contribute to the absence of differences in the relaxant effect between large and small coronary arteries. From the present results, KRN2391 and nicorandil are expected to have beneficial effects in the redistribution of coronary blood flow.

In conclusion, the effects of KRN2391 as well as those of nicorandil were equipotent in both large and small coronary arteries. This result of KRN2391 is considered to be due to its dual mechanism of action as a nitrate and a potassium-channel opener. Thus, this profile of KRN2391 is expected to cause appropriate distribution of coronary blood flow based on dilation of large coronary arteries. However, further evaluation in-vivo is required.

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