range similar to those tested in this study, exerts marked behavioural changes in rodents; these behavioural effects are antagonized by nifedipine and may reflect a functional consequence of the region-specific changes in dopaminergic and 5-HT-ergic neurotransmission via an interaction with dihydropyridine-binding sites within the CNS (Janis et al 1984; Bolger et al 1985).

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Dissimilarity in the mechanisms of action of KRN2391, nicorandil and cromakalim in canine renal artery

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Abstract—In the present study, we examined the mode of action of KRN2391 (*N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate) in isolated canine renal artery compared with those of nicorandil and cromakalim. KRN2391 (10^{-8}–3×10^{-5} M), nicorandil (10^{-7}–3×10^{-4} M) and cromakalim (10^{-8}–3×10^{-5} M) relaxed renal arteries contracted by 25 mM KCl in a concentration-dependent manner. KRN2391-induced relaxation was inhibited by methylene blue (10^{-5} M) and glibenclamide (10^{-6} M). Nicorandil-induced relaxation was inhibited by methylene blue, but not by glibenclamide. The concentration-relaxation curve for cromakalim displayed a rightward parallel shift in the presence of glibenclamide. In the control observation, KRN2391 and nicorandil also produced full relaxation, but cromakalim did not. The present results suggest that KRN2391 acts as both a nitrate and a potassium channel opener, and nicorandil acts only as a nitrate and only in canine renal artery.

KRN2391, *N*-cyano-*N'*-(*N*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate, is a novel agent possessing vasodilating action (Kashiwabara et al 1991). As shown in Fig. 1, KRN2391 has a nitrate moiety in its structure. KRN2391-induced relaxation was inhibited by both a guanylate cyclase inhibitor and a K^+-channel blocker in rat isolated aorta (Kashiwabara et al 1991). However, in canine large coronary artery, this relaxation was inhibited by a guanylate cyclase inhibitor, but not by a K^+-channel blocker (Fukata et al 1991). In canine cranial mesenteric artery, both a guanylate cyclase inhibitor and a K^+-channel blocker antagonized the relaxation induced by KRN2391 (Fukata et al 1991). Kingsbury et al (1991) also reported that KRN2391 predominantly behaved as a K^+-channel opener in resistive coronary arterioles of dogs. Though KRN2391 has a dual mechanism of action as a nitrate and as a K^+-channel opener, its mechanism of action is thought to depend on the segment of vascular bed and the type of blood vessel. Therefore, it is of interest whether KRN2391 shows its action as a K^+-channel opener or as a nitrate in renal artery,

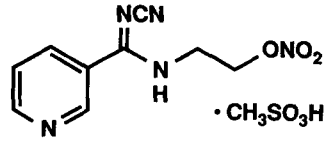

FIG. 1. Chemical structure of KRN2391.

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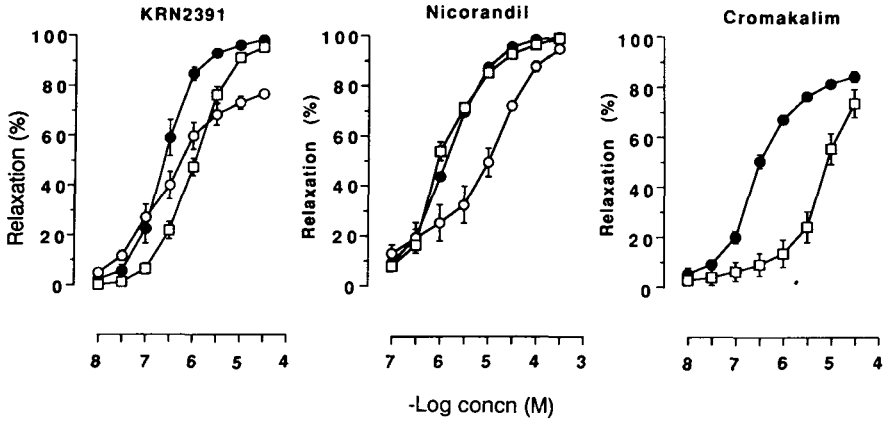

FIG. 2. Concentration-relaxation curves for KRN2391 and nicorandil in canine renal arteries contracted by 25 mM KCl in the absence (●) and the presence of methylene blue (10^{-5} M, ○) or glibenclamide (10^{-6} M, □). The effect of cromakalim was examined in the absence and the presence of glibenclamide. Each point is the mean ± s.e.m. of 4–5 experiments.

another important artery. In the present study, we examined the mechanism of action of KRN2391 in isolated renal artery of dog.

Materials and methods

Preparation. Renal arteries were obtained from beagles of either sex, 7·8–11·3 kg, anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, intravenously). The arteries were cut into 2–3 mm long ring segments, and the endothelium was removed by rubbing. These preparations were mounted on stainless steel wires in organ baths filled with 10 mL of Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25·0, glucose 10·0, at 37°C aerated with 95% O_2–5% CO_2. The artery rings were stretched by an initial resting tension of 1·0 g. After an initial equilibration period of 120 min, the artery rings 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 added cumulatively. Methylene blue and glibenclamide were added 20 min before the arteries were contracted by 25 mM KCl. Isometric tension was measured with a mechanical transducer (Nihon Kohden, TB-611T), and was recorded on an ink-writing recorder (TOA, FBR-252A).

Drugs. KRN2391, nicorandil and cromakalim were synthesized in our laboratory. Methylene blue and glibenclamide were purchased from Wako and Sigma, respectively. KRN2391 was dissolved in double-distilled water at 10^{-2} M, nicorandil in 0·1 M HCl at 5×10^{-2} M and cromakalim in dimethylsulphoxide at 10^{-2} M. Glibenclamide and methylene blue were also dissolved at 10^{-3} M in dimethylsulphoxide and at 10^{-2} M in double distilled water, respectively. These stock solutions were diluted to the appropriate concentration in Krebs–Henseleit solution.

Data analysis. The relaxation caused by the test drugs was expressed as a percentage of the maximum relaxation obtained by addition of 10^{-4} M papaverine at the end of each experiment. Data are given mean ± s.e.m. The mean EC50 was determined from the concentration-relaxation curves which were fitted by linear regression. Differences were considered significant when $P < 0.05$ using Student's *t*-test and analysis of variance for multiple comparisons. When multiple comparisons were made with a single control, Dunnett's test was used to determine significance level.

Results

KRN2391 (10^{-8}–3×10^{-5} M), nicorandil (10^{-7}–3×10^{-4} M) and cromakalim (10^{-8}–3×10^{-5} M) produced a concentration-dependent relaxation in renal arteries contracted by 25 mM KCl (Fig. 2). KRN2391 and nicorandil produced almost full relaxation at concentrations of their maximum effects. However, cromakalim-induced relaxation was about 84·9% of the complete relaxation even at concentrations showing its maximum effect.

The concentration-relaxation curves for KRN2391 and nicorandil shifted rightward in the presence of 10^{-5} M methylene blue (Fig. 2). The EC50 values of KRN2391 and nicorandil in the presence of methylene blue were significantly higher than that of the control (Table 1).

Glibenclamide (10^{-6} M) had no effect on the concentration-relaxation curve for nicorandil (Fig. 2). The concentration-relaxation curves for KRN2391 and cromakalim in the presence of glibenclamide shifted to the right (Fig. 2). The EC50 value of cromakalim in the presence of glibenclamide was significantly higher than that of control (Table 1). However, the magnitude of

Table 1. EC50 values for the relaxant effects of KRN2391, nicorandil and cromakalim against 25 mM KCl-induced contraction in the absence and presence of methylene blue or glibenclamide.

	EC50 values ($\times 10^{-6}$ M)		
	KRN2391	Nicorandil	Cromakalim
Control	0·29 ± 0·08	1·25 ± 0·09	0·60 ± 0·10
Methylene blue (10^{-5} M)	1·18 ± 0·50*	6·85 ± 2·49*	
Glibenclamide (10^{-6} M)	1·13 ± 0·17*	1·24 ± 0·08	9·36 ± 3·26*

Values are mean ± s.e.m. of 4–5 experiments. *$P < 0.05$ compared with the corresponding control value.

shift of the concentration-relaxation curve and EC50 value for KRN2391 in the presence of glibenclamide was lower than that for cromakalim.

Discussion

The present results show that the mechanism of action of KRN2391 is different from that of nicorandil in canine renal artery. The relaxation induced by KRN2391 was antagonized by both methylene blue, an inhibitor of soluble guanylate cyclase (Gruetter et al 1981), and glibenclamide, an inhibitor of ATP-sensitive K^+-channels (Schmid-Antomarchi et al 1987). However, the inhibitory effect of glibenclamide on the relaxation induced by KRN2391 was weaker than that by cromakalim which is a K^+-channel opener (Weir & Weston 1986; Yanagisawa et al 1990), because the magnitude of shift of the concentration-relaxation curve and EC50 value for KRN2391 was smaller than that for cromakalim. These results suggest that both nitrate-like and K^+-channel opening actions contribute to KRN2391-induced relaxation in canine renal artery. The relaxation induced by nicorandil was also antagonized by methylene blue, but not by glibenclamide, suggesting that nicorandil acts like a nitrate alone.

We have already reported that the mechanism of action of KRN2391 differs with the type of blood vessel (Fukata et al 1991). KRN2391-induced relaxation in canine large coronary artery was inhibited by methylene blue but not by glibenclamide, while in cranial mesenteric artery both methylene blue and glibenclamide inhibited the relaxation by KRN2391 (Fukata et al 1991). Thus, KRN2391 behaved as a nitrate in canine large coronary artery and as both a nitrate and a K^+-channel opener in canine cranial mesenteric artery. On the other hand, nicorandil predominantly behaved as a nitrate without showing any action as a K^+-channel opener in both canine large coronary (Satoh et al 1991) and cranial mesenteric arteries (unpublished data). However, both KRN2391 and nicorandil appear to show K^+-channel opening action rather than nitrate-like action in resistive arterioles. Yoneyama et al (1990) reported that the increase in coronary blood flow caused by nicorandil was inhibited by glibenclamide in isolated blood-perfused canine papillary muscle, suggesting its K^+-channel opening action in dilating resistive coronary arterioles. Furthermore, Kingsbury et al (1991) also demonstrated that KRN2391 as well as nicorandil predominantly behaved as a K^+-channel opener in the same blood-perfused preparation. Therefore, although the mechanism of action of KRN2391 is similar to that of nicorandil in resistive arterioles, there seems to be a difference between KRN2391 and nicorandil in the mechanism of action on the large segment of various vascular beds. KRN2391 is thought to act as both a K^+-channel opener and a nitrate in canine mesenteric and renal arteries, and as a nitrate in canine large coronary arteries. However, the mechanism of action of nicorandil seems to be nitrate-like in all large segments of coronary, mesenteric and renal vascular beds. This insufficient relaxation in the presence of methylene blue is also observed in cranial mesenteric artery in which KRN2391 shows both a nitrate-like and a K^+-channel opening action (Fukata et al 1991).

In the present study, KRN2391 and nicorandil produced almost full relaxation at their maximum effects in isolated canine renal arteries contracted by 25 mM KCl. Cromakalim produced insufficient relaxation at its maximum effect. This different result between nicorandil and cromakalim is similar to that obtained in canine large coronary artery (Satoh et al 1991). The insufficient relaxation caused by cromakalim is thought to be supported by the observation that the relaxant effect of cromakalim on KCl-induced contraction depends on the concentration of KCl (Yanagisawa et al 1990). The full relaxation induced by KRN2391 may be observed by its nitrate-like action. Indeed, KRN2391 produced the full relaxation in the presence of glibenclamide despite showing the insufficient relaxation even at 3×10^{-5} M in the presence of methylene blue.

In conclusion, although the modes of action of KRN2391 and nicorandil depend on the segment of vascular bed and the type of blood vessel, KRN2391 acts as both a K^+-channel opener and a nitrate, and nicorandil behaves as a nitrate in canine renal arteries.

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