Differential vasodilator properties of KRN2391, cromakalim, nitroglycerin and nifedipine in rabbit isolated femoral artery and vein

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- 1 The selectivity for artery and vein of KRN2391, cromakalim, nitroglycerin and nifedipine was examined in isolated femoral artery and vein preparations of the rabbit.
- 2 All drugs produced a concentration-dependent relaxation in both femoral artery and vein.
- 3 Nitroglycerin was more potent in femoral vein than in femoral artery at all concentrations. The EC₅₀ value obtained in the vein was about 14 times smaller than that obtained in artery.
- 4 Cromakalim and nifedipine were almost equipotent on both vascular preparations. Cromakalim at the highest concentration (10⁻⁵ M) produced 88 and 78% relaxation in femoral artery and vein, respectively. The maximum relaxation induced by nifedipine (10⁻⁶ M) was less than 50% in both preparations.
- 5 KRN2391 was active at a lower concentration in the vein than in the artery and its maximum relaxation at 10⁻⁵ M was about 90% in both preparations.
- 6 Glibenclamide (10⁻⁶ M) inhibited the vasorelaxation caused by KRN2391 in both artery and vein. Methylene blue (10⁻⁵ M) also inhibited the relaxant action of KRN2391 but this action was slight in the artery.
- 7 These results suggest that KRN2391 and nitroglycerin are more potent in the vein than in the artery and cromakalim and nifedipine are equipotent in both. It is considered that the relaxation induced by low concentrations of KRN2391 reflects predominantly its action as a nitrate and that at high concentrations it acts as a K⁺ channel opener in addition to its nitrate action. The different vascular selectivities of these drugs are thought to relate to the differences in their mechanisms of action in vascular smooth muscle.

Keywords: KRN2391; nitroglycerin; cromakalim; nifedipine; rabbit femoral artery; rabbit femoral vein

Introduction

KRN2391, N-cyano-N'-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethansulphonate, is a new vasodilator which has a nitrate moiety in its chemical structure. With respect to its mechanism of action, it has been reported to possess both a K⁺ channel opening action and a nitrate action (Kashiwabara *et al.*, 1991; Okada *et al.*, 1991; Ishibashi *et al.*, 1992). In *in vivo* experiments on anaesthetized dogs, KRN2391 produced a preferential increase in coronary blood flow in comparison with other peripheral blood flows (Ogawa *et al.*, 1993). In addition, KRN2391 has been reported to induce an increase in oxygen supply through increases in coronary blood flow and decreases in myocardial oxygen consumption in dogs (Ogawa *et al.*, 1992). Thus, KRN2391 is thought to be a vasodilator selective for the coronary vascular bed with both a K⁺ channel opening action and a nitrate action. Recently, Kasai *et al.* (1993) have shown a potent anti-vasoconstrictor effect of KRN2391 in porcine isolated coronary arteries contracted by noradrenaline, acetylcholine, 5-hydroxytryptamine, thromboxane A₂ (using a stable analogue U46619) and endothelin-1 which are considered to be the cause of coronary vasospasm (Vanhouste & Houston, 1985). From these profiles of KRN2391, it is possible that it could be useful in therapy of ischaemic heart disease such as angina pectoris.

At present, nitrates and Ca²⁺ channel blockers are extensively used as antianginal drugs. Although the coronary dilator actions of nitrates and Ca²⁺ channel blockers contribute to their beneficial effects, the influences of these drugs

on preload or afterload also play an important role in their antianginal effects. The finding that vasodilators produce either a reduction in preload or afterload is associated with their selectivity for vein or artery, i.e. vasodilators selective for vein and venules such as nitroglycerin produce a reduction in preload (Gotanda *et al.*, 1989), while vasodilators relatively selective for artery such as Ca²⁺ channel blockers produce a potent reduction in afterload (Sakanashi *et al.*, 1986; 1988). Therefore, a study concerning the selectivity of KRN2391 on vein and artery was considered to be important in analysing its effect on systemic circulation *in vivo*. In the present study, we examined the properties of KRN2391 compared with those of nitroglycerin and nifedipine in femoral artery and vein of the rabbit. Cromakalim, a K⁺ channel opener (Weir & Weston, 1986; Coldwell & Howlett, 1987), was also examined because a K⁺ channel opening action partly contributes to the vasodilator mechanism of KRN2391.

Methods

Preparations

Femoral arteries and veins were excised from male rabbits (New Zealand White, 2.7–3.5 kg) that had been anaesthetized with ether, and killed by exsanguination. Immediately after excision, these vessels were placed in Krebs-Ringer solution of the following composition (mM): NaCl 112, KCl 4.7, CaCl₂ 2.2, NaHCO₃ 25, MgCl₂ 1.2, KH₂PO₄ 1.2 and glucose 14. Adherent fat and connective tissue were cleaned from the vessels, and rings of tissues (each 3 mm long) were prepared. The endothelium was removed by rubbing the intimal surface

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with a stainless steel rod. Each preparation was mounted in an organ bath filled with 10 ml Krebs-Ringer solution. This was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Isometric tension was recorded on a pen-writing recorder (FBR-252A, TOA Electronics, Tokyo, Japan) via a transducer (TB-611T, Nihon Koden, Tokyo, Japan). An initial resting tension of 0.5 g and 0.2 g was applied to the arterial and venous preparations, respectively. These resting tensions were chosen by referring to Nakajima & Nosaka (1983). After an initial equilibration period of 120 min, the preparations were contracted by 10⁻⁶ and 10⁻⁵ M noradrenaline in arteries and veins, respectively. These concentrations of noradrenaline were determined as those producing submaximum contractions (see Results). When the preparations had reached stable tension, each test drug was then added noncumulatively to the organ bath according to the method of Nakajima & Nosaka (1983). Glibenclamide (10⁻⁶ M) and methylene blue (10⁻⁵ M) were added to the bath 20 min before the addition of noradrenaline when their effects on KRN2391-induced relaxation were to be evaluated.

Drugs

KRN2391 and cromakalim were synthesized at Kirin Brewery Pharmaceutical Research Laboratory. (-)-Noradrenaline bitartrate and papaverine hydrochloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). Glibenclamide, methylene blue and nifedipine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Nitroglycerin was used from commercially available ampoules (Millisrol, Nippon Kayaku, Tokyo, Japan). Cromakalim (5 × 10⁻³ M) and nifedipine (10⁻² M) were dissolved in dimethyl sulphoxide, and glibenclamide was prepared in dimethyl sulphoxide/10% KHCO₃/twice distilled water (10:5:85). These solutions were diluted in twice distilled water. All other drugs were dissolved in twice distilled water.

Statistical analysis

Data are presented as mean ± s.e.mean. Relaxation responses caused by the test drugs are expressed as a percentage of the maximum relaxation attainable at the end of each experiment by the addition to the bath of 10⁻⁴ M papaverine. Differences were evaluated for significance (*P* < 0.05) by Student's *t* test for unpaired data for single comparisons. The concentration of a drug which caused a 50% response (EC₅₀ value) with 95% confidence limits was calculated by the probit method.

Results

Figure 1 shows concentration-response curves for noradrenaline in the femoral artery and vein. The effect of noradrenaline was more potent in the femoral artery than in the vein. The EC₅₀ value was $7.18 \pm 0.08 \times 10^{-8}$ M in artery (*n* = 6) and $7.75 \pm 3.53 \times 10^{-7}$ M in vein (*n* = 7). The concentrations of noradrenaline chosen to induce submaximum contractions were 10⁻⁶ M and 10⁻⁵ M in femoral artery and vein, respectively. The developed tension produced by these concentrations of noradrenaline was 4.8 ± 0.33 g in the artery (*n* = 6) and 0.42 ± 0.07 g in the vein (*n* = 7).

Figure 2 shows concentration-response curves for the test drugs in the femoral artery and vein. Nitroglycerin over the concentration range 10⁻⁹–10⁻⁵ M produced a concentration-dependent relaxation in femoral artery and vein (Figure 2a). The threshold concentration of nitroglycerin was lower in the vein than in the artery. The activity of nitroglycerin was greater in the vein than in the artery and at 10⁻⁷ and 10⁻⁶ M, the % relaxation value in the vein was significantly greater than that in the artery. Cromakalim (10⁻⁷–10⁻⁵ M) and nifedipine (10⁻¹⁰–10⁻⁶ M) also produced a concentration-dependent relaxation in the femoral artery and vein (Figure 2b and c). Cromakalim showed similar activity in the artery

Figure 1 Concentration-response curves for noradrenaline in rabbit isolated femoral artery (O) and vein (●). Ordinate scale: % contraction expressed as a percentage of the response at 10⁻⁴ M noradrenaline. Each value is presented as a mean ± s.e.mean derived from 6–7 experiments.

and vein at 10⁻⁷ M but at 10⁻⁶ M the % relaxation value was significantly greater in artery than that in vein. KRN2391 studied over the concentration-range 10⁻⁹–10⁻⁵ M produced relaxation in femoral artery and vein in a concentration-dependent manner (Figure 2d). At 10⁻⁸ and 10⁻⁷ M, the vasodilator activity of KRN2391 was significantly greater in the vein than in the artery.

Table 1 summarizes the maximum relaxation of each drug in artery and vein. The maximum response of each drug was not different between artery and vein.

Table 2 shows the EC₅₀ value of each drug. The EC₅₀ values of KRN2391 and nitroglycerin in artery were greater than those in vein. In contrast, the value of cromakalim in artery was smaller than that in vein. Because the relaxation at 10⁻⁶ M nifedipine did not reach 50% in both vascular preparations (Figure 2c and Table 1), the EC₅₀ values of nifedipine could not be calculated.

Figure 3 shows the effects of glibenclamide (10⁻⁶ M) and methylene blue (10⁻⁵ M) on the KRN2391-induced relaxation in the femoral artery and vein. Table 2 also shows the EC₅₀ values in the presence of glibenclamide or methylene blue. Glibenclamide inhibited the vasorelaxation caused by KRN2391 and the concentration-response curves similarly shifted to the right both in artery and vein. The EC₅₀ values in the presence of glibenclamide were about 11 times greater than those in the absence of glibenclamide in both vascular preparations. Methylene blue also antagonized the relaxant action of KRN2391. The concentration-response curve in the vein shifted to the right at all concentrations of KRN2391, but the action of KRN2391 obtained in the artery was clearly inhibited by methylene blue only at 10⁻⁶ M KRN2391. The EC₅₀ values with methylene blue were 2.7 and 6.4 times greater than those without methylene blue in artery and vein, respectively.

Discussion

In the present study, at low concentrations, KRN2391 produced a greater vasodilatation in the femoral vein than in the femoral artery, whereas, at high concentrations it produced equal vasodilatation in artery and vein. Since the vasorelaxant activity of KRN2391 is reported to be based on both a nitrate action and a K⁺ channel opening action (Kashiwabara *et al.*, 1991; Okada *et al.*, 1991; Ishibashi *et al.*, 1992), the properties of KRN2391 observed in the present

Table 1 The maximum relaxation induced by KRN2391, nitroglycerin, cromakalim and nifedipine against contractions produced by noradrenaline in isolated femoral artery and vein of the rabbit

	Maximum relaxation (%)	
	Artery	Vein
Nitroglycerin (10 ⁻⁵ M)	51.73 ± 7.67	60.78 ± 2.51
Cromakalim (10 ⁻⁵ M)	88.20 ± 3.77	77.50 ± 0.65
Nifedipine (10 ⁻⁶ M)	44.48 ± 0.92	41.58 ± 5.28
KRN2391 (10 ⁻⁵ M)	90.18 ± 2.58	86.50 ± 2.26

Each value is the mean ± s.e.mean of 4–7 experiments.

Table 2 EC₅₀ values for the relaxation effects of KRN2391, nitroglycerin and cromakalim against contractions induced by noradrenaline

	EC ₅₀ (10 ⁻⁷ M)	
	Artery	Vein
Nitroglycerin	111 (48.6–361)	7.95 (3.59–21.8)
Cromakalim	3.81 (2.65–5.56)	8.65 (5.40–14.7)
KRN2391	4.02 (2.74–6.00)	2.10 (1.37–3.27)
KRN2391 + glibenclamide	43.6 (31.0–62.0)	24.5 (16.4–36.9)
KRN2391 + methylene blue	10.7 (7.45–15.4)	13.4 (8.11–22.5)

Each value is calculated by the probit method from the results of 4–8 experiments.

The numbers in parentheses indicate 95% confidence limits.

study appear to reflect this dual mechanism of action. The experiments with blocking agents provided evidence of these properties of KRN2391. Vasorelaxation induced by KRN2391 in both artery and vein was antagonized by glibenclamide, a pharmacological antagonist of K⁺ channel openers (Cavero *et al.*, 1989; Eltze, 1989). On the other hand, methylene blue, an inhibitor of soluble guanylate cyclase (Grutter *et al.*, 1981), inhibited the response of the vein more than that of the artery. Therefore, it is considered that the relaxation induced by low concentrations reflects predominantly its action as a nitrate and that at high concentrations reflects its actions as a K⁺ channel opener in addition to a nitrate action.

Nitroglycerin produced more potent vasodilatation in the femoral vein than in the femoral artery. These results with nitroglycerin are in good agreement with those of earlier studies in similar preparations from the same species (Nakajima & Nosaka, 1983; Toyoda *et al.*, 1986). The guanosine 3':5'-cyclic monophosphate (cyclic GMP) increased by activating guanylate cyclase acts as a mediator of nitrates including nitroglycerin (Kukovetz *et al.*, 1979; Itoh *et al.*, 1985). The next step is thought to involve hyperpolarization of cell membranes (Ito *et al.*, 1978), inhibition of Ca²⁺ influx

Figure 2 Effects of nitroglycerin (a), cromakalim (b), nifedipine (c) and KRN2391 (d) on the contractions induced by noradrenaline in femoral artery (○) and vein (●). Ordinate scale: % relaxation expressed as a percentage of the response at 10⁻⁴ M papaverine. Each value is presented as a mean ± s.e.mean derived from 4–7 experiments. **P* < 0.05, ***P* < 0.01: significant difference from the corresponding value for the artery.

Figure 3 Effects of glibenclamide (10^{-6} M, ●) and methylene blue (10^{-5} M, ■) on KRN2391-induced relaxation of rabbit femoral artery (a) and vein (b) precontracted with noradrenaline. The control concentration-response curves (○) are the same as those in Figure 2d. Ordinate scale: % relaxation expressed as a percentage of the response to 10^{-4} M papaverine. Each value is presented as a mean $\pm$ s.e.mean derived from 4–8 experiments.

(Thorens & Haeusler, 1979), an increase in Ca^{2+} extrusion (Itoh *et al.*, 1985), Ca^{2+} sequestration (Imai & Kitazawa, 1981) and a reduction in the amount of myosin light chain phosphorylation (Murad, 1986). The difference in development of these processes may be associated with the different sensitivity to nitroglycerin between artery and vein. Axelsson *et al.* (1982) also proposed that nitrates, including nitro-

glycerin, induce vasodilatation by interacting with the sulphydryl group on a nitrate 'receptor'. Therefore, another possible explanation of the different sensitivity may be that there is a difference in the quantity of sulphydryl groups between artery and vein.

The present study showed that cromakalim at high concentrations produced more potent vasodilatation of the femoral artery than of the femoral vein. K^{+} channel openers such as cromakalim, are thought to act by increasing the outward K^{+} current by opening K^{+} channels and thereby causing hyperpolarization of cell membranes (Cook, 1988). This hyperpolarization induces a reduction in intracellular free Ca^{2+} through the inhibition of voltage-dependent Ca^{2+} channels and receptor-operated Ca^{2+} channels (Cook, 1988). Recently, in addition to these mechanisms, K^{+} channel openers have been reported to reduce the release of Ca^{2+} from intracellular stores through inhibition of inositol-1,4,5-trisphosphate production (Ito *et al.*, 1991). There may be a difference in the number of K^{+} channels between the femoral artery and vein.

The relaxation induced by nifedipine was less than 50% even at its maximum concentration in femoral artery and vein. In contrast, KRN2391 and cromakalim produced about 78–90% relaxation in both preparations. These results indicate that the remaining contraction by noradrenaline in the presence of nifedipine is due to Ca^{2+} release from intracellular storage sites. As mentioned above, the vasodilator mechanism of K^{+} channel openers is thought to be based on the inhibitions of extracellular Ca^{2+} influx and release of Ca^{2+} from intracellular storage sites, triggered by hyperpolarization of the cell membranes. Therefore, KRN2391 and cromakalim seem to be able to inhibit the remaining contraction which cannot be inhibited by nifedipine. Nitroglycerin also produced less than 60% relaxation even at 10^{-5} M. However, nitroglycerin-induced relaxation did not reach a plateau in the femoral artery and vein. In the present study, we did not examine the vasorelaxant activity of nitroglycerin at higher concentrations because a higher concentration of nitroglycerin could not be obtained. If more than 10^{-5} M nitroglycerin is used, it may induce further relaxation in femoral artery and vein.

In summary, the present results suggest that the vasorelaxant activity of nitroglycerin was greater in the femoral vein than in the femoral artery, while cromakalim and nifedipine were equipotent in both vascular smooth muscle preparations. In addition, the properties of KRN2391 at low concentrations are similar to those of nitroglycerin but at high concentrations its effects seem to reflect a combination of actions of nitroglycerin and cromakalim. The differences in vasorelaxant activity of KRN2391, nitroglycerin, cromakalim and nifedipine between the femoral artery and vein are thought to be based on their different mechanisms of action in vascular smooth muscle.

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