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Differential antagonism by Bay K 8644 of vasodilator effects of nifedipine, diltiazem, nicorandil and nitroglycerin in dog femoral circulation

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1 The modification by Bay K 8644 of the vasodilator effects of nifedipine, diltiazem, nicorandil and nitroglycerin was investigated in the femoral arterial bed of anaesthetized dogs.

2 The right femoral artery was cannulated and its arterial bed was perfused with autologous blood at a constant pressure slightly higher than the mean systemic arterial blood pressure. Bay K 8644 was infused intra-arterially (i.a.) and the 4 vasodilators were injected i.a. as bolus doses.

3 The vasodilator effects of nifedipine (0.3–10 nmol), diltiazem (0.01–1 μmol), nicorandil (0.1–10 μmol) and nitroglycerin (0.3–100 nmol) were all suppressed by infusions of Bay K 8644 (3–100 nmol min^{-1}).

4 The dose-response curve of nifedipine was shifted parallel to the right by the infusion of Bay K 8644 and the dose-ratio was the greatest of the 4 drugs.

5 The dose-response curve of diltiazem was also shifted to the right by Bay K 8644. However, the dose-ratio was far smaller than that of nifedipine.

6 The vasodilator effect of nicorandil was not antagonized as much by Bay K 8644 as that of nitroglycerin. This less effective antagonism of nicorandil by Bay K 8644 can be explained if nicorandil, which although structurally a nitrate, can in addition cause relaxation of vascular smooth muscle by hyperpolarizing the membrane which would result in Bay K 8644 being less effective.

Introduction

Bay K 8644, a dihydropyridine calcium agonist, increases the systemic arterial blood pressure when administered intravenously (i.v.) to dogs (Schramm *et al.*, 1983a,b) and decreases blood flow through the coronary (Wada *et al.*, 1985; Ishii *et al.*, 1986), femoral, mesenteric (Ishii *et al.*, 1986) and renal (Ishii *et al.*, 1986; Ogawa & Ono, 1986) arterial beds (i.e. resistance vessels) in dogs when administered intra-arterially (i.a.). However, in rabbit isolated aortae (Schramm *et al.*, 1983a,b) and mesenteric arteries (Kanmura *et al.*, 1984) (i.e. conductance vessels) Bay K 8644 produces contractions only when these tissues are at least partly depolarized by high potassium. In these isolated arterial preparations Bay K 8644 has been found to augment preferentially contractions produced by high potassium rather than those elicited by noradrenaline (Schramm *et al.*, 1983a,b; Kanmura *et al.*, 1984). Based on these observations, it has been suggested that Bay K 8644 produces contractions by promoting the calcium influx when voltage-dependent calcium channels are operative (Schramm *et al.*, 1983a,b; Kanmura *et al.*, 1984). Moreover, the contractile effects of Bay K 8644 on these isolated arterial preparations have been found to be inhibited competitively by dihydropyridine calcium antagonists and non-competitively by non-dihydropyridine calcium antagonists (Schramm *et al.*, 1983a,b; Kanmura *et al.*, 1984).

In previous experiments on the dog saphenous arterial bed (a resistance vessel preparation), Bay K 8644 was found to augment the vasoconstrictor effect of noradrenaline (Goto *et al.*, 1985). This, taken together with the direct constrictor effect of Bay K 8644 on resistance vessels, implies that, in smooth muscle cells of resistance vessels, Bay K 8644 is also able to exert its effect when receptor-operated calcium channels are operative. In other words, it is likely that conductance vessels and resistance vessels respond to Bay K 8644 differently. Therefore, it was of interest to see whether or not the interactions between Bay K 8644 and the two types of calcium antagonists, i.e., dihydropyridine and non-dihydropyridine types, on

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resistance vessels are similar to those observed in conductance vessels. In the present study, we investigated the interactions of Bay K 8644 and the two calcium antagonists, nifedipine (a dihydropyridine) and diltiazem (non-dihydropyridine), in the dog femoral arterial bed. Furthermore, the interactions of Bay K 8644 and the two nitrate vasodilators, nicorandil and nitroglycerin, were studied to see whether or not the effects of the drugs were inhibited by Bay K 8644 differently, because nicorandil, unlike other nitrates, is also known to relax vascular smooth muscle by hyperpolarizing the membrane through a mechanism which increases membrane potassium conductance (Furukawa *et al.*, 1981; Itoh *et al.*, 1981; Weir & Weston, 1986).

Methods

Experiments were performed on mongrel dogs of either sex, weighing 7–18 kg. The dogs were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) and given heparin calcium (500 u kg^{-1} i.v.). The right femoral artery was cannulated and perfused with blood from the left carotid artery. The blood was delivered by means of a peristaltic pump (Harvard Apparatus, 1215) and the perfusion pressure was kept constant, at a level slightly higher than the mean systemic arterial blood pressure, by placement of a Starling pneumatic resistor through which excess blood was conducted to the left femoral vein. Blood flow through the right femoral artery was measured with an electromagnetic flow meter (Nihon Kohden, MFV-2100) and recorded on charts by use of a rectilinear recorder (San-ei, Rectihoriz-8K). The systemic arterial blood pressure, heart rate and perfusion pressure of the right femoral artery were also recorded.

Nifedipine (0.3–10 nmol), diltiazem (0.01–1 μmol), nicorandil (0.1–10 μmol) and nitroglycerin (0.3–100 nmol) were injected into the right femoral artery in a constant volume of 0.1 ml over 5 s. Bay K 8644 (3–100 nmol min^{-1}) was infused i.a. at a constant rate (0.1 ml min^{-1}) by means of an infusion pump (Harvard Apparatus, 600-000).

Responses of the right femoral arterial bed to these vasodilators were measured as changes (increases) in blood flow through this arterial bed. After the control dose-response curve for a vasodilator had been determined, the lowest dose of Bay K 8644 was infused and the dose-response curve for this vasodilator was obtained. This procedure was repeated with increasing doses of Bay K 8644. The determination of the vasodilator dose-response curve was begun about 5 min after the start of the Bay K 8644 infusion. Each dog received only one vasodilator.

Experimental values are expressed as mean ± s.e.mean. Dose-response curves for the vasodilator effects of the 4 drugs before and during infusion of Bay K 8644 were approximated by linear regressions. For each vasodilator, parallelism of the regression lines between before and during infusion of Bay K 8644 was tested by analysis of covariance (Snedecor & Cochran, 1967). When P values were less than 0.05, the lines were considered to be non-parallel. For each curve, the dose that produced 40 ml min^{-1} increase in blood flow (ED 40 ml min^{-1}) was calculated from the regression line to determine the dose-ratios.

Bay K 8644 (Bayer), nifedipine (Bayer), diltiazem hydrochloride (Tanabe), nicorandil (Chugai) and nitroglycerin (0.05% aqueous solution in ampoule, Nippon Kayaku) were used. Bay K 8644 was dissolved in 99.5% ethanol. Nifedipine was dissolved in 15% ethanol, 35% polyethyleneglycol 400 and water. Diltiazem and nicorandil were dissolved in 0.9% w/v NaCl solution (saline). These compounds were diluted to the desired concentrations with saline.

Results

The basal blood flow through the right femoral artery was 29.4 ± 2.3 ml min^{-1} (n = 28) at an average perfusion pressure of 142 ± 3.5 mmHg. Systolic and diastolic arterial blood pressures were 212 ± 6.6 mmHg and 113 ± 3.7 mmHg, respectively, and heart rate was 169 ± 4.6 beats min^{-1}.

Before infusions of Bay K 8644 (control), single bolus injections of nifedipine (0.3–10 nmol), diltiazem (0.01–1 μmol), nicorandil (0.1–10 μmol) or nitroglycerin (0.3–100 nmol) into the right femoral artery produced comparable increases in blood flow through the artery and, at the highest dose, the increases amounted to about 3 times the basal value (Figure 1). With higher doses of these drugs, a transient decrease in systemic arterial blood pressure and a transient increase in heart rate were observed. The solvents of these drugs, in amounts required to dissolve the doses used, had little effects on these variables.

Infusions of Bay K 8644 (3–100 nmol min^{-1}) into the right femoral artery produced a sustained, and dose-dependent, decrease in blood flow (Figure 2). The highest dose decreased the blood flow to about a quarter of the basal level. The systemic arterial blood pressure and heart rate were scarcely affected by the infusion. With infusion of the solvent of Bay K 8644 (ethanol) at corresponding concentrations, blood flow through the right femoral artery, systemic arterial blood pressure and heart rate remained unaffected.

The vasodilator effects of the 4 drugs were all suppressed and their dose-response curves were shifted in a rightward direction by infusions of Bay 8644 (3–100 nmol min^{-1}; Figure 1). Parallel shifts of the dose-response curves approximated by regression

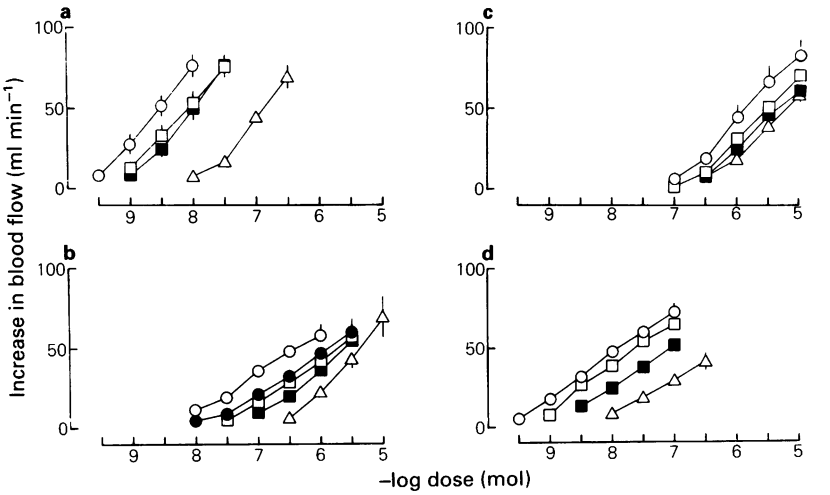

Figure 1 Dose-response curves for increases in blood flow through the right femoral artery to (a) nifedipine ($n = 7$), (b) diltiazem ($n = 9$), (c) nicorandil ($n = 6$) and (d) nitroglycerin ($n = 6$), before (○) and during infusion of Bay K 8644 (●) 3 nmol min^{-1}, (□) 10 nmol min^{-1}, (■) 30 nmol min^{-1}, (△) 100 nmol min^{-1}. Vertical lines represent s.e.mean or, where absent, are contained within the symbol.

lines occurred for nifedipine and nicorandil. However, the dose-response curve for diltiazem, obtained during the infusion of 100 nmol min^{-1} Bay K 8644 was not parallel to, but steeper than the control. Likewise, the dose-response curve for nitroglycerin determined with the infusion of 100 nmol min^{-1} Bay K 8644 was not parallel to, but less steep than the control.

Tolerance to nitroglycerin is known to develop when applied repeatedly at short time intervals (Needleman & Johnson, 1975; Nabata *et al.*, 1981). To test this possibility, similar experiments were carried out in which nitroglycerin was administered during infusions of the solvent (ethanol) at the same intervals as used with infusions of Bay K 8644 (Figure 3).

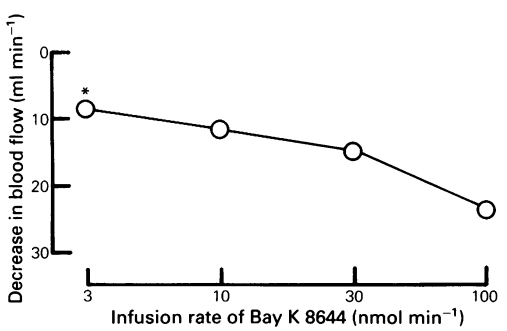

Figure 2 Infusion rate-response curve for the effect of Bay K 8644 ($n = 28$; *$n = 8$) in the right femoral artery. Bay K 8644 was infused and blood flow measured through the artery.

Analysis of the data by analysis of variance revealed that the effect of nitroglycerin with each infusion was identical. Therefore, the suppression of the effect of nitroglycerin observed during the infusion of Bay K 8644 was considered to be the effect of Bay K 8644 itself.

For each vasodilator, doses that increased the femoral blood flow by 40 ml min^{-1} (ED 40 ml min^{-1})

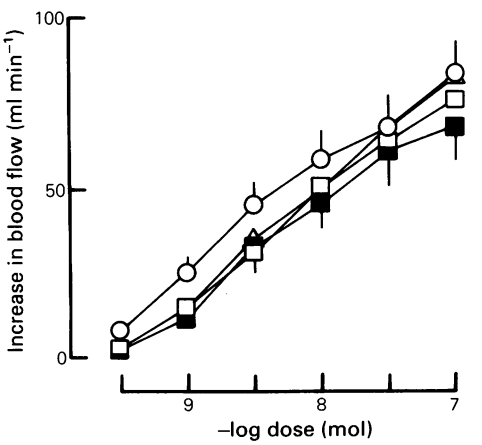

Figure 3 Dose-response curves for the effect of nitroglycerin on blood flow through the right femoral artery ($n = 6$), before (○) and during an infusion of ethanol (□) 3.3%, (■) 10%, (△) 33.3%. Vertical lines represent s.e.mean.

Table 1 A comparison of the dose-response curve parameters (ED 40 ml min^{-1} and dose-ratio) for the vasodilator effects of nifedipine, diltiazem, nicorandil and nitroglycerin during infusion of Bay K 8644

		Infusion of Bay K8644 (nmol min^{-1})		
	Control	*10*	*30*	*100*
Nifedipine				
ED 40 ml min^{-1} (nmol)	1.65 (1.28–2.13)	4.48 (3.84–5.22)	5.53 (3.75–8.14)	82.5 (73.2–92.9)
Dose-ratio		2.72	3.35	50.0
Diltiazem				
ED 40 ml min^{-1} (μmol)	0.16 (0.10–0.28)	0.73 (0.56–0.94)	1.03 (0.53–2.01)	2.13 (1.40–3.25)*
Dose-ratio		4.56	6.44	13.3
Nicorandil				
ED 40 ml min^{-1} (μmol)	0.81 (0.52–1.29)	1.61 (1.52–1.70)	2.32 (1.69–3.20)	3.20 (2.07–4.95)
Dose-ratio		1.99	2.86	3.95
Nitroglycerin				
ED 40 ml min^{-1} (nmol)	5.84 (5.26–6.49)	11.3 (8.31–15.5)	36.9 (29.3–46.4)	282.7 (208.8–382.7)**
Dose ratio		1.93	6.32	48.4

ED 40 ml min^{-1} is the dose that increased the femoral blood flow by 40 ml min^{-1}. Confidence limits for 95% are given in parentheses. *Regression line is steeper than control. **Regression line is less steep than control.

were determined from the regression lines and the dose-ratios calculated are presented in Table 1.

Discussion

In the present experiments, Bay K 8644 suppressed vasodilator responses of the dog femoral arterial bed (resistance vessels) to nifedipine, diltiazem, nicorandil and nitroglycerin. Even at the highest infusion rate of Bay K 8644 (100 nmol min^{-1}), the maximum responses to nifedipine, diltiazem and nicorandil were not different from the control. Thus, dose-ratios were obtainable for these 3 vasodilators from the doses that produced a 40 ml min^{-1} increase in flow of the femoral circulation. The order of magnitude of dose-ratios at the infusion rate of 100 nmol min^{-1} of Bay K 8644 was as follows: nifedipine > diltiazem > nicorandil. The dose-ratio of nitroglycerin was not compared to those of the others because the drug failed to retain the maximum response in the dose range examined.

In conductance vessels, it has been demonstrated that Bay K 8644 inhibits the effects of dihydropyridine calcium antagonists competitively and those of non-dihydropyridine calcium antagonists non-competitively (Schramm *et al.*, 1983a,b; Kanmura *et al.*, 1984). Thus, it was of interest to see whether similar interactions also occur in resistance vessels. Under the conditions of the present experiments, it is impossible to say whether the interaction of drugs observed is competitive or not, because the concentrations of drugs at the site of action may vary and the effects probably do not reflect the equilibrium. Nevertheless, the dose-ratio of nifedipine obtained with an infusion of Bay K 8644 at a rate of 100 nmol min^{-1} was about 4 times greater than that of diltiazem. This suggests that nifedipine and diltiazem interact with Bay K 8644 differently in resistance vessels, too.

Bay K 8644 also suppressed the effects of nicorandil and nitroglycerin. However, the effect of nitroglycerin was reduced to a greater extent by Bay K 8644 than that of nicorandil. Tolerance is well known to develop when nitroglycerin is applied repeatedly and the reduction in effect seen in the present experiments might have reflected the tolerance to the drug. Therefore, six doses of nitroglycerin (0.3–100 nmol) were repeated 4 times with infusions of the solvent (ethanol) instead of Bay K 8644. However, the effects of nitroglycerin for each determination were identical. Therefore, it is safe to conclude that the suppression of the effect of nitroglycerin was due to Bay K 8644 and that nicorandil and nitroglycerin interact with Bay K 8644 differently. It has been demonstrated that the vasodilator effects of the two nitrates on the monkey coronary arterial bed constricted by acetylcholine are different (Satoh *et al.*, 1984). The decreased coronary blood flows were readily overcome by nicorandil (Satoh *et al.*, 1984) but only to a small extent by nitroglycerin (Taira *et al.*, 1983). The present results taken together with the previous results on the monkey coronary circulation, suggest a different mode of vasodilator action of the two nitrates. Nicorandil, although structurally a nitrate, is known to hyperpolarize membranes of smooth muscle cells of arteries and thereby exert its vasodilator effect (Furukawa *et al.*, 1981; Itoh *et al.*, 1981; Weir & Weston, 1986). However, nitroglycerin does not modify the membrane electrical properties (Ito *et al.*, 1980). Thus, it is likely that the vasoconstrictor effect of Bay K 8644, which is voltage-dependent, was counteracted by the hyperpolarizing action of nicorandil and, hence, dif-

ferential effects on nicorandil and nitroglycerin were observed.

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