Br. J. Pharmac. (1985), **85**, 913–916

Bay K 8644, a dihydropyridine calcium agonist, augments vasoconstrictor responses to endogenous and exogenous noradrenaline in the peripheral vasculature of the dog

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1 The effect of Bay K 8644 (a substance known to increase calcium influx through the voltage-dependent calcium channel) on vasoconstrictor responses of resistance vessels to endogenous and exogenous noradrenaline (NA) was investigated in pentobarbitone-anaesthetized dogs which had also undergone spinal anaesthesia and bilateral vagotomy and received atropine.

2 In these dogs the saphenous arterial bed was perfused at fixed flow rates with autologous blood to give perfusion pressure close to the systemic blood pressure.

3 Electrical stimulation (3–30 Hz) of the saphenous nerve and single intra-arterial (i.a.) injections of noradrenaline (NA, 0.03–3 μg) produced an increase in perfusion pressure (vasoconstriction) in a frequency- and a dose-dependent manner, respectively.

4 Intra-arterial infusions of Bay K 8644 (3 and 10 μg min^{-1}) *per se* produced no significant change in perfusion pressure. However, these infusions augmented vasoconstrictor responses to both saphenous nerve stimulation (endogenous NA) and i.a. NA (exogenous NA).

5 These results suggest that augmentation by Bay K 8644 of vasoconstrictor responses of resistance vessels to endogenous and exogenous NA is probably due to promotion of the calcium influx through calcium channels closely associated with α-adrenoceptors in smooth muscle cells there.

Introduction

Methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methyl)-pyridine-5-carbaxylate (Bay K 8644), unlike many other dihydropyridine derivatives, produces an increase in systemic blood pressure, and positive inotropy and chronotropy (Schramm *et al.*, 1983a,b). Bay K 8644, however, produced contractions of rabbit isolated aortae only when they were depolarized by high potassium (Schramm *et al.*, 1983a,b). Moreover, Bay K 8644 augmented contractions of rabbit aortae produced by high potassium but not by noradrenaline (NA) (Schramm *et al.*, 1983b). Based on these observations Schramm *et al.* (1983a,b) have put forward a hypothesis that Bay K 8644 promotes the calcium influx when voltage-dependent calcium channels are operative. Kanmura *et al.* (1984) have obtained essentially similar results in smooth muscle cells of the rabbit mesenteric artery, and supported the hypothesis of Schramm *et al.* (1983a,b). In the experiments by Kanmura *et al.* (1984) Bay K 8644 enhanced contractions produced by high concentrations of NA. Thus, it appeared that the results obtained by Kanmura *et al.* (1984) with NA differed from those by Schramm *et al.* (1983b). However, a high concentration of NA was thought to depolarize smooth muscle cells of the rabbit mesenteric artery to such an extent that voltage-dependent calcium channels were operative (Kanmura *et al.*, 1984). Thus, there is no disagreement on the mechanism of action of Bay K 8644 on vascular smooth muscle between these two groups of investigators. However, it should be noted that the results which have favoured the hypothesis of Schramm *et al.* (1984a,b) have been obtained from experiments on conductance vessels. Therefore, it is not yet settled whether Bay K 8644 produces vasoconstriction of resistance vessels by a mechanism similar to that found in conductance vessels.

NA is thought to produce vasoconstriction at least partly by increasing calcium influx through calcium channels presumed to be closely associated with α-adrenoceptors in vascular smooth muscle (α-adren-

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oceptor-operated calcium channels). Therefore, it was of great interest to know whether Bay K 8644 would augment vasoconstrictor responses of resistance vessels to NA. A previous study (Satoh *et al.*, 1985) has demonstrated that the saphenous arterial bed of the dog responds to electrical stimulation of the saphenous nerve and i.a. injections of NA by vasoconstriction and both responses involve α-adrenoceptors of vascular smooth muscle. Therefore, in the present study, we investigated the effect of Bay K 8644 on vasoconstrictor responses of resistance vessels to saphenous nerve stimulation (endogenous NA) and i.a. NA exogenous NA) in the saphenous arterial bed of the dog.

Methods

Five mongrel dogs of either sex weighing 11 to 17 kg were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.v.), given heparin sodium (500 u kg^{-1} i.v.) and artificially ventilated. Dibucaine (9 mg) was injected into the cisterna magna to induce spinal anaesthesia. Both vagi were cut and atropine sulphate (1 mg kg^{-1} i.v.) and nadolol (1 mg kg^{-1} i.v.) were given intravenously.

The saphenous artery on one side was perfused with blood from the femoral artery on the other side at a constant flow rate by a peristaltic blood pump (Harvard Apparatus, 1210). The flow rate was adjusted initially to give a perfusion pressure close to the systemic blood pressure and fixed at this value.

The distal end of the cut saphenous nerve was stimulated with a train of rectangular electric pulses of 1 ms duration at a frequency of 3, 10, 20 and 30 Hz for 30 s. Stimulus voltage ranged from 20 to 25 V.

NA solutions were injected singly into the saphenous artery in a constant volume of 30 μl for 4 s with microsyringes. Bay K 8644 was infused i.a. at a constant rate (0.1 ml min^{-1}), and the infusion was started 2 min before saphenous nerve stimulation or NA injection.

Responses of the saphenous arterial bed to saphenous nerve stimulation and NA injection were measured as changes in perfusion pressure by means of a pressure transducer (Nihon Kohden, MP-4T) and the responses before (control) and during the infusion of Bay K 8644 were compared.

(−)-Noradrenaline base (Fluka, Buchs SG) was dissolved in 0.01 N HCl solution at a concentration of 1 mg ml^{-1}. Bay K 8644 was dissolved in 99.5% ethanol at a concentration of 1 mg ml^{-1}. The stock solutions thus made were diluted to the desired concentrations with 0.9% NaCl solution.

Experimental values were presented in terms of means ± s.e.mean. Significant differences between mean values were evaluated by the use of Student's *t* test. *P* values less than 0.05 were considered significant.

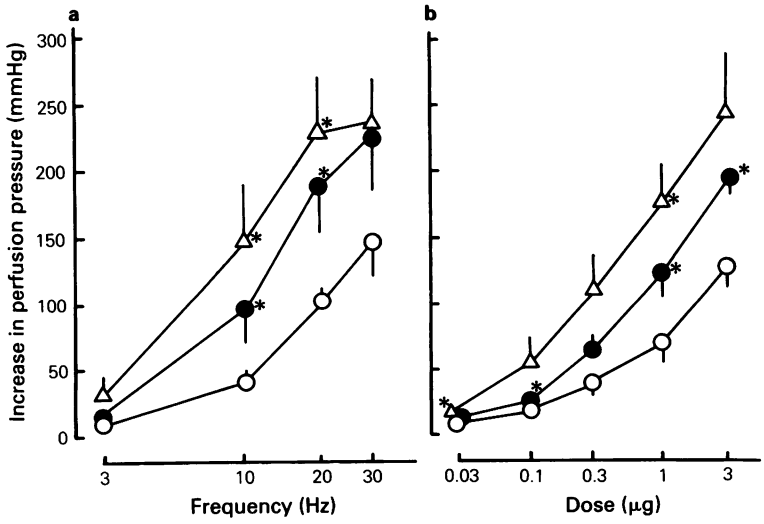

Figure 1 Frequency- and dose-response curves for increase in perfusion pressure of the saphenous arterial bed to saphenous nerve stimulation (a) and noradrenaline (NA) injected to the saphenous artery (b), before (○) and during infusion of Bay K 8644 (●, 3 μg min^{-1}; △, 10 μg min^{-1}). Data points represent means from 5 dogs; s.e.mean shown by vertical lines. * $P < 0.05$ when compared with the increase in perfusion pressure before infusion of Bay K 8644 at corresponding frequencies of saphenous nerve stimulation (a) and corresponding same doses of NA (b).

Results

In 5 anaesthetized dogs which underwent spinal anaesthesia, bilateral vagotomy and received atropine, the mean systemic blood pressure was 70.2 ± 6.5 mmHg. In these animals perfusion pressure of the saphenous arterial bed averaged 72.8 ± 2.6 mmHg under conditions of perfusion at fixed flow rates ranging 4.5–13 ml min^{-1}. Under these conditions, saphenous nerve stimulation (3–30 Hz) and single injections of NA (0.03–3 μg) into the saphenous artery produced an increase in perfusion pressure (vasoconstriction) in a frequency- and a dose-dependent manner, respectively (Figure 1). Both vasoconstrictor responses were reproducible over a period of about 2 h, the time required for the experiments described below.

Infusions of Bay K 8644 (3 and 10 μg min^{-1}) into the saphenous artery *per se* changed neither perfusion pressure (72.6 ± 2.1 mmHg at 3 μg min^{-1} and 72.2 ± 2.3 mmHg at 10 μg min^{-1} against 72.8 ± 2.6 mmHg in control) nor systemic blood pressure (69.2 ± 6.4 mmHg at 3 μg min^{-1} and 69.7 ± 7.1 mmHg at 10 μg min^{-1} against 70.2 ± 6.5 mmHg in control). Nevertheless, these infusions augmented markedly vasoconstrictor responses to both saphenous nerve stimulation and NA injection. The augmentation of vasoconstriction was greater at 10 μg min^{-1} than at 3 μg min^{-1}, except for vasoconstriction in response to nerve stimulation at 30 Hz which reached nearly a maximum at 3 μg min^{-1}. Thus, frequency-response curves to saphenous nerve stimulation and dose-response curves to NA shifted upwards.

Discussion

In the present experiments Bay K 8644 augmented vasoconstrictor responses of resistance vessels to endogenous and exogenous NA. The augmentation was dependent upon the dose of Bay K 8644 and was seen with small responses to small doses of i.a. NA and to low frequency stimulation of the saphenous nerve. Thus, the present results are at variance both with those obtained by Schramm *et al.* (1983a,b) in rabbit isolated aortae and those obtained by Kanmura *et al.* (1984) in rabbit isolated mesenteric arteries (cf. Introduction). The question arises whether vascular smooth muscle cells in resistance vessels, responsible for vasoconstriction observed in the present experiments, are depolarized by endogenous and exogenous NA to such an extent that voltage-dependent calcium channels are operative. A similar question has been addressed as to the mechanism of action of calcium channel blockers in antagonizing vasoconstrictor responses to NA of peripheral vascular beds, since calcium channel blockers have been claimed to be far more effective on voltage-dependent calcium channels than on receptor-operated ones (Triggle, 1981). However, doubt was cast by Cauvin *et al.* (1982) on this conceptual framework of the mechanism of action of calcium channel blockers. They demonstrated that in rabbit mesenteric resistance vessels diltiazem, a calcium channel blocker, antagonized vasoconstriction and ^{45}Ca influx in response to NA more effectively than those to high potassium. However, the effects of diltiazem in rabbit aortae were opposite to those observed in rabbit mesenteric resistance vessels (Van Breemen *et al.*, 1981). Therefore, it is likely that in smooth muscle cells of resistance vessels, calcium channel promotors like Bay K 8644 are also able to exert their effect when receptor-operated calcium channels are operative as are calcium channel blockers. In other words, Bay K 8644 augmented vasoconstrictor responses of resistance vessels probably by promoting the calcium influx through calcium channels thought to be closely associated with α-adrenoceptors on smooth muscle cells.

Bay K 8644 has opposite effects to calcium channel blockers, as it has been shown to augment pressor responses to α_2-adrenoceptor agonists to a far greater extent than those to α_1-adrenoceptor agonists in pithed rats (Wilffert *et al.*, 1984). Accordingly it has been claimed that α_2-adrenoceptors are more closely associated with calcium channels than α_1-adrenoceptors (Wilffert *et al.*, 1984). A previous study (Satoh *et al.*, 1985) showed that in the dog saphenous arterial bed, vasoconstrictor responses to saphenous nerve stimulation involve α_1-adrenoceptors to a greater extent than α_2-adrenoceptors on vascular smooth muscle and the opposite relation holds in vasoconstrictor responses to i.a. NA. In the present experiments vasoconstrictor responses to saphenous nerve stimulation and i.a. NA were almost equally augmented by Bay K 8644. Thus, it is likely that in peripheral vasculature of the dog both α_1- and α_2-adrenoceptors are equally associated with calcium channels.

It is also of interest that, in the present experiments, Bay K 8644 augmented almost equally vasoconstrictor responses to both endogenous and exogenous NA. It is thought that the release of NA from adrenergic nerve terminals by nerve impulses depends on calcium influx through voltage-dependent calcium channels there (Blaustein, 1979). Thus, it is presumed that Bay K 8644 would facilitate this process which would lead to much more enhanced vasoconstrictor responses to endogenous NA than to exogenous NA. However, this did not occur in the present experiments. Therefore, it can be concluded that the effect of Bay K 8644 is

overwhelmingly postsynaptic. This is consistent with the results that nifedipine, a dihydropyridine calcium channel blocker, is far less active on presynaptic sites than on postsynaptic sites in noradrenergic neuroeffector transmission (Starke & Schümann, 1973).

The authors are grateful to Prof. Dr med. F. Hoffmeister, Bayer AG, Pharmakologisches Institut, Wuppertal, F.R.G. for kindly supplying Bay K 8644.

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(Received February 11, 1985.
Revised March 16, 1985.
Accepted March 21, 1985)