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Bay K 8644-induced changes in the ECG pattern of the rat and their inhibition by antianginal drugs

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1 The effects of intracarotid administration of Bay K 8644 on the ECG pattern along with their reversal by antianginal drugs were investigated in anaesthetized rats.

2 Intracarotid injections of Bay K 8644 (0.5–50.0 μgkg^{-1}) produced a dose-related transient increase in systemic blood pressure.

3 The pressor response was accompanied by ST segment elevation (0.5–10.0 μgkg^{-1}), ST segment depression concomitant with the occurrence of arrhythmias (20.0 μgkg^{-1}), or A-V block (50.0 μgkg^{-1}).

4 ST segment elevation reached its maximal value within 15 s and could be observed for 30–240 s.

5 The increase in blood pressure was immediate (within 5 s) and short lasting (30–120 s). After the initial increase it returned to control levels (0.5–20.0 μgkg^{-1}) or dropped below (50.0 μgkg^{-1}).

6 The ST segment elevation caused by 5.0 μgkg^{-1} Bay K 8644 (submaximal dose) was blocked by antianginal drugs (e.g. nitroglycerin, nifedipine and diltiazem) and by the peripheral benzodiazepine receptor antagonist PK 11195. However, the pressor response was not blocked by any of the drugs used.

7 ST segment elevation (or depression) induced by intracarotid administration of Bay K 8644 provides a useful tool for the evaluation of potential antianginal drugs.

Introduction

Bay K 8644, a dihydropyridine derivative, acts as a calcium channel activator by increasing calcium influx through voltage-dependent calcium channels (Schramm *et al.*, 1983a; Hess *et al.*, 1984). Subsequently, it was demonstrated that Bay K 8644 produced marked vasoconstriction of isolated arteries and veins (Schramm *et al.*, 1983b; Su *et al.*, 1984; Gopalakrishnan *et al.*, 1985; Mikkelsen *et al.*, 1985), positive inotropic and chronotropic effects in various cardiac preparations (Satoh *et al.*, 1984; Finet *et al.*, 1985; Wada *et al.*, 1985) and a decrease in coronary blood flow (Schramm *et al.*, 1983a; Wada *et al.*, 1985; Ishii *et al.*, 1986). Administration of Bay K 8644 to intact animals, produced an increase in arterial blood pressure and a positive inotropic effect accompanied by coronary vasospasm and the occurrence of arrhythmias (Schramm *et al.*, 1983a,b; Gross *et al.*, 1985).

As a reduction in coronary blood flow may lead to cardiac ischaemia and predispose to ECG abnormalities, the present study was designed to investigate the effects of intracarotid administration of Bay K 8644 on the ECG pattern and examine its response to various drugs. In addition to the clinically used antianginal drugs (e.g., nitroglycerin, nifedipine and diltiazem) we examined PK 11195, a potent antagonist of the peripheral benzodiazepine receptor that has been shown to decrease arrhythmias induced by myocardial ischaemia in the dog (Mestre *et al.*, 1985a). Phentolamine, an α-adrenoceptor antagonist and a potent hypotensive drug as well as atropine, a muscarinic cholinoceptor antagonist, were also studied. We demonstrated that intracarotid administration of Bay K 8644 elicited changes in the ECG pattern and that these alterations could be inhibited by antianginal drugs and PK 11195, but not by phentolamine or atropine.

Methods

Experimental procedure

Male Sprague-Dawley rats (Charles River, England, 450–500 g) were anaesthetized with sodium pentobarbitone (25 $mgkg^{-1}$, i.p.). Catheters were placed in the right femoral artery and vein for monitoring systemic arterial blood pressure and for the administration of drugs, respectively. Blood pressure was measured by

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means of a Statham blood pressure transducer coupled to a polygraph recorder (Model MDP-4B, Narco Bio-Systems). Lead I of the ECG was recorded using an electrocardiograph (model 1500B, Hewlett Packard); heart rate was derived from the ECG tracings. The right carotid artery was cannulated (PE-50, 1000 u ml^{-1} of heparin) and the cannula pushed forward to the region of the coronary ostium as described by Sakai *et al.* (1981). Cardiac ischaemia was induced by intracarotid administration of Bay K 8644.

Experimental values are expressed as mean ± s.e.mean. Statistical analyses were performed by use of Student's paired *t* test (two-tailed).

Drugs and chemicals

Bay K 8644 (methyl 1, 4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) was a gift from Dr M. Schramm (Bayer, West Germany) and PK 11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-isoquinotine-3-carboxamide) a gift from Dr G. Le Fur (Pharmuka Laboratories, France).

Atropine sulphate, diltiazem hydrochloride and nifedipine were purchased from Sigma. Nitroglycerin and phentolamine were obtained from Teva Pharmaceuticals (Israel) and Ciba-Geigy corporation (USA), respectively. All drugs, except for Bay K 8644, PK 11195 and nifedipine, were dissolved in saline (0.9% NaCl solution). Bay K 8644 and nifedipine were dissolved in absolute ethanol and diluted with saline. PK 11195 was dissolved in a mixture of Emulphor EL-620 (GAF, West Germany) and ethanol (1:1) and diluted with saline. These agents were injected in volumes not exceeding 100 µl and containing less than 7 µl of ethanol. One hundred µl of vehicle alone had no effect on blood pressure or ECG pattern.

Results

Effects of Bay K 8644

Intracarotid administration of Bay K 8644 (0.5–50.0 μgkg^{-1}) affected both blood pressure and ST segment amplitude. The pressor response to Bay K 8644 increased in a dose-dependent manner at a dose range of 0.5–10.0 μgkg^{-1}; this effect was decreased at doses higher than 20.0 μgkg^{-1} (Figure 1). The elevation of blood pressure was discernible within less than 5 s after the injection of Bay K 8644 and lasted for 30–120 s. At a dose range of 0.5–20.0 μgkg^{-1} blood pressure returned to control levels, whereas at a dose of 50.0 μgkg^{-1} the pressor response was followed by a short hypotensive phase and death (data not shown).

The effect of Bay K 8644 on the ST segment amplitude was evident within 5–10 s after injection,

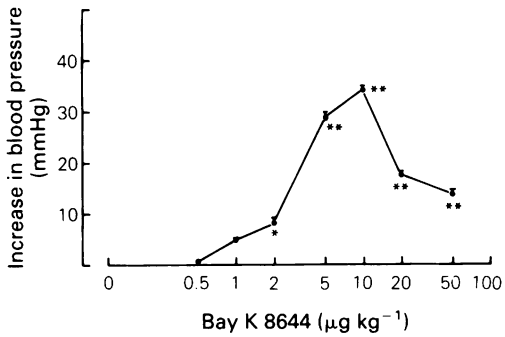

Figure 1 Dose-related increase in arterial blood pressure induced by Bay K 8644. Results are expressed in mmHg as mean. ($n = 3$) change from control value (115 ± 1.9 mmHg, $n = 32$); vertical lines indicate s.e.mean. The following doses were determined in larger groups of animals: 5.0 μgkg^{-1} ($n = 12$), 10.0 μgkg^{-1} ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.001$.

reached its maximal value within 15 s and lasted for 30–240 s. Figure 2 shows typical ECG tracings of Bay K 8644-induced ST segment alterations. As demonstrated in this figure, doses of 5.0 ($n = 12$) and 10.0 ($n = 6$) μgkg^{-1} Bay K 8644 caused ST segment elevation (one animal out of the latter group developed ST segment depression). ST segment depression was observed in all the animals exposed to 20.0 ($n = 3$) and 50.0 ($n = 3$) μgkg^{-1} Bay K 8644. Arrhythmias preceded the ST segment depression in one of the animals administered Bay K 8644, 20 μgkg^{-1} (data not shown). All the animals treated with 50.0 μgkg^{-1} developed atrioventricular block and died within one min. In order to quantify the ST segment amplitude, the degree of S wave elevation (or depression) was measured in mV units relative to the isoelectric potential (Vergona *et al.*, 1984). The dose-related changes in S wave amplitude are depicted in Figure 3. This figure clearly demonstrates that Bay K 8644 produced a bidirectional effect on the ST segment amplitude. At doses up to 10.0 μgkg^{-1} ST segment elevation was obtained whereas at higher doses ST segment depression was recorded.

No effect on heart rate was detectable at doses below 5.0 μgkg^{-1}, while with this and with higher dose levels bradycardia was noted. Control heart rate was 382.5 ± 4.3 beats min^{-1} ($n = 26$); in the presence of Bay K 8644 heart rate decreased to 347.5 ± 4.5, 324.1 ± 6.2 and 310.0 ± 1.8 beats min^{-1} at 5.0, 10.0 and 20.0 μgkg^{-1}, respectively.

Influence of drugs on the effects of Bay K 8644

A single dose level of Bay K 8644 (5.0 μgkg^{-1}) was used for assessing the ability of drugs to inhibit the changes in blood pressure and ST segment amplitude caused by

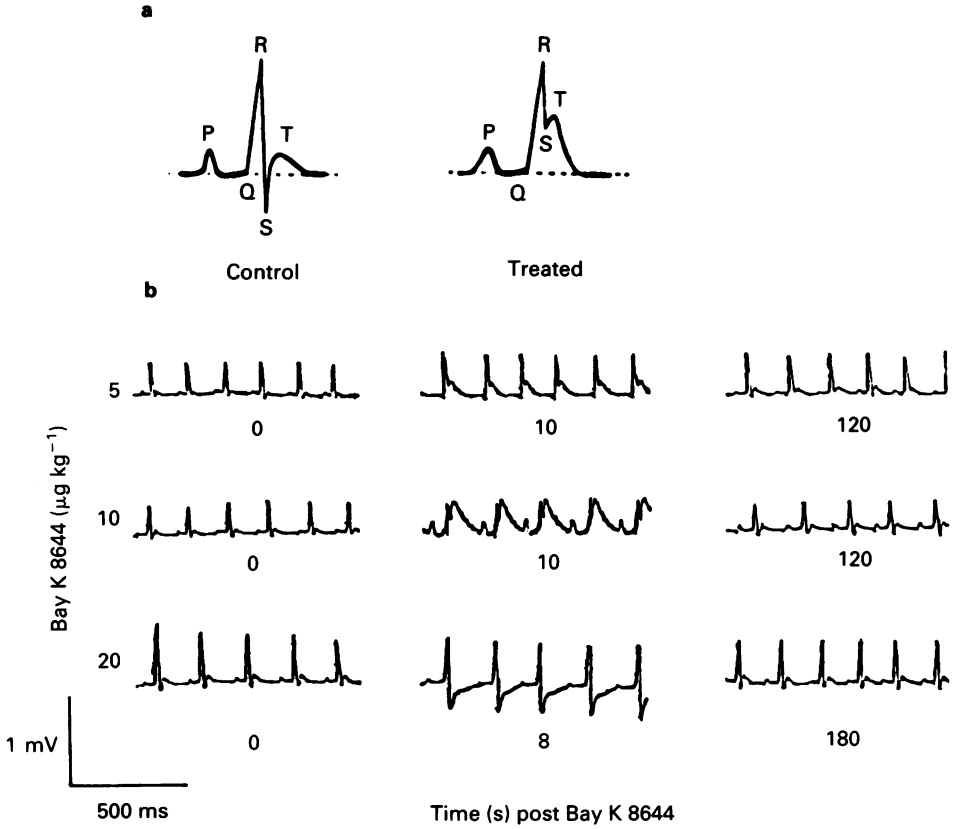

Figure 2 (a) Schematic illustrations of ECG tracings before and after the introduction of Bay K 8644. S wave elevation (or depression) was derived by measuring the distance of the S wave from the isoelectric potential (broken line). (b) Representative tracings of Lead I of the ECG were obtained in anaesthetized rats at the indicated times following the introduction of various doses of Bay K 8644 (via the carotid artery).

this compound. This dose was chosen as it produced a submaximal effect on both ST segment amplitude and blood pressure. Drugs were injected at doses outlined previously (Sakai *et al.*, 1981; Vergona *et al.*, 1984), and at different time intervals before the administration of Bay K 8644. The latter reflects the earliest time to peak effect estimated experimentally (data not shown). All drugs tested, except for PK 11195, produced a hypotensive response. Although blood pressure recovered spontaneously thereafter, a residual decrease was still evident at the time of Bay K 8644 administration. The extent of this decrease, presented as % of control values: 6.5 ± 1.3 (nitroglycerin, $50\,\mu gkg^{-1}$), 10.0 ± 1.7 (nifedipine, $50\,\mu gkg^{-1}$), 20.3 ± 1.8 (nifedipine, $100\,\mu gkg^{-1}$), 38.5 ± 4.7 and 33.7 ± 3.8 (phentolamine, 15 and 60 min post injection, respectively). In the case of nitroglycerin ($25\,\mu gkg^{-1}$), diltiazem and atropine blood pressure reached control levels before the introduction of Bay K 8644.

The ability of the drugs tested to block the ST segment elevation and pressor response caused by Bay K 8644 ($5.0\,\mu gkg^{-1}$) is summarized in Table 1. Phentolamine and atropine failed to block the ECG changes or the pressor response produced by Bay K 8644. Conversely, the antianginal drugs nitroglycerin, nifedipine and diltiazem as well as PK 11195 effectively blocked the ST segment elevation produced by the calcium channel agonist. However, these drugs did not affect the bradycardia elicited by $5.0\,\mu gkg^{-1}$ Bay K 8644.

Preliminary experiments with higher doses of Bay K 8644 (10.0 and $20\,\mu gkg^{-1}$) have indicated that nitroglycerin ($50\,\mu gkg^{-1}$) and diltiazem ($500\,\mu gkg^{-1}$) completely blocked the ST segment alterations evoked by the calcium channel activator.

Table 1 Inhibition of Bay K 8644-induced S wave elevation and pressor response by various compounds

Compound (time)[a]	*Dose* (μgkg^{-1})[b]	*Blockade of Bay K 8644-induced* *S wave elevation*	*Pressor response*[c]
Nitroglycerin	25	42.6 ± 5.8*	5.5 ± 2.8
(1)	50	100.0**	12.0 ± 6.1
Nifedipine	50	58.3 ± 6.9*	10.3 ± 5.5
(15)	100	73.3 ± 10.9*	6.3 ± 4.4
Diltiazem	250	94.6 ± 4.4*	7.7 ± 3.9
(15)	500	100.0**	8.0 ± 4.3
PK 11195	100	100.0**	0.0
(1)			
Phentolamine	5000	0.0	7.9 ± 2.8
(15)			
(60)		0.0	6.4 ± 3.1
Atropine	5000	0.0	0.0
(15)			

Bay K 8644 ($5.0\,\mu gkg^{-1}$) was injected via the carotid artery to anesthetized rats. Various compounds were introduced at the indicated doses and time intervals. Results are expressed as % (mean ± s.e.mean) change ($n = 3$) of control values obtained in the presence of Bay K 8644 alone.

[a] Time (min) before Bay K 8644 administration ($5.0\,\mu gkg^{-1}$).

[b] Drugs were administered intravenously except for PK 11195 (administered via the carotid artery).

[c] These values were not significantly different from controls.

* $P < 0.025$, ** $P < 0.001$.

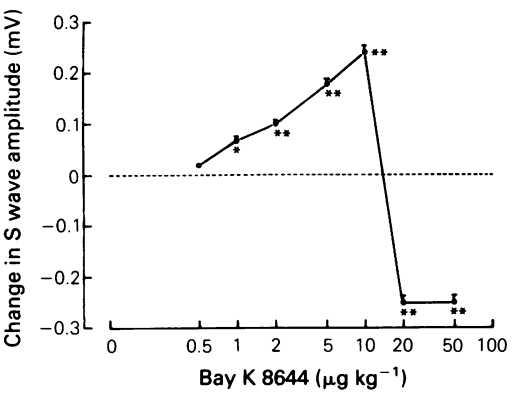

Figure 3 Dose-related changes in S wave amplitude induced by Bay K 8644 injected into the carotid artery. Results are expressed in mV as mean ($n = 3$) change from control value of S wave amplitude (-0.052 ± 0.004 mV, $n = 32$); vertical lines indicate s.e.mean. The following doses were determined in larger groups of animals: $5.0\,\mu gkg^{-1}$ ($n = 12$), $10.0\,\mu gkg^{-1}$ ($n = 5$). * $P < 0.05$, ** $P < 0.005$.

Discussion

ST segment elevation is generally considered to be a sensitive measure of cardiac ischaemia (Brezinski *et al.*, 1986). This study demonstrates that intracarotid administration of Bay K 8644 elicits transient ST segment alterations that might represent cardiac ischaemia. Two mechanisms may be involved in the Bay K 8644-induced cardiac ischaemia, namely, decreased myocardial oxygen supply due to coronary vasospasm and increased myocardial metabolic demands due to a positive inotropic effect. In fact, Bay K 8644 has been shown to produce coronary vasoconstriction and to decrease coronary blood flow (Schramm *et al.*, 1983a; Taira *et al.*, 1984; Wada *et al.*, 1985). These effects were more pronounced than its inotropic, chronotropic and dromotropic effects (Wada *et al.*, 1985). The effective blockade of Bay K 8644-induced ischaemia by nitroglycerin, diltiazem and nifedipine correlates well with their antianginal activity (Needleman *et al.*, 1985). Unlike nitroglycerin, a known coronary vasodilator (Abrams, 1980), the other two drugs exert their action via inhibition of voltage-dependent calcium channels in both cardiac and coronary tissues (Braunwald, 1982). We may thus suggest that while nitroglycerin acts primarily as a coronary vasodilator, the beneficial effect of diltiazem and nifedipine may be ascribed to both coronary vasodilatation and to their negative inotropic effect. PK 11195, a typical antagonist of peripheral benzodiazepine receptors, has been demonstrated to inhibit indirectly myocardial voltage-dependent calcium channels (Mestre *et al.*, 1985b; 1986). This compound was devoid of any effect on blood pressure and thus it seems that the observed antianginal effect resides in its negative inotropism. These observations demonstrate that blockade of Bay K 8644-induced cardiac ischaemia can be achieved by either coronary

vasodilatation or negative inotropism.

Biphasic activity of Bay K 8644 has been observed previously by several groups. For example, in isolated heart preparations high doses of Bay K 8644 produced a negative inotropic effect (Schramm *et al.*, 1983a; Franckowiak *et al.*, 1985). Likewise, in isolated blood vessels Bay K 8644 exhibited a biphasic effect on the contractile force (Dube *et al.*, 1985; Mikkelsen, 1985). Franckowiak *et al.* (1985) have demonstrated that the (−)-enantiomer of Bay K 8644 possesses vasoconstrictor and positive inotropic effects whereas high concentrations of its antipode produce negative inotropic and vasodilator effects. Thus, according to these authors our results (Figure 1) may be accounted for by the use of the racemic mixture of Bay K 8644.

None of the compounds used in this study effectively blocked the pressor response and the bradycardia induced by Bay K 8644. Lefer *et al.* (1986) also noted that even continuous infusion of the calcium channel antagonists, nitrendipine and nisoldipine, at doses which decrease blood pressure by 30 mmHg could only partially block the pressor response due to Bay K 8644. In addition, the results presented here (Table 1) and by Lefer *et al.* (1986) demonstrate that the Bay K 8644-induced increase in blood pressure was not antagonized by phentolamine. As mentioned above, PK 11195 had no effect on blood pressure but still completely reversed ST segment alterations (Table 1). Therefore, the present study indicates that blockade of Bay K 8644-induced pressor response is not essential for the inhibition of the observed ischaemia.

In contrast to the animal model of cardiac ischaemia described by Sakai *et al.* (1981), the present study is based on direct activation of the contractile machinery rather than indirect action via activation of muscarinic receptors by methacholine. Moreover, Vergona *et al.* (1984) have cautioned against the use of methacholine-induced ischaemia for the screening of antianginal drugs as it may identify compounds with antimuscarinic activity (e.g. atropine). In our hands atropine was found to be ineffective even at a relatively high dose level (5 $mgkg^{-1}$) (Table 1) which causes a hypotensive response due to an α-adrenoceptor blocking effect (Abraham *et al.*, 1981).

However, the results we obtained in this study are not decisive enough to differentiate between the proposed mechanisms of action of Bay K 8644. More studies are needed to delineate further these mechanisms on the cardiovascular system. Our findings suggest that Bay K 8644-evoked cardiac ischaemia in the rat provides a tool for the screening of potential antianginal drugs.

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