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Electropharmacological effects of berberine on canine cardiac Purkinje fibres and ventricular muscle and atrial muscle of the rabbit

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1 Conventional microelectrode techniques were used for intracellular recordings of the transmembrane electrical potentials, the effects of berberine were studied on canine cardiac Purkinje and ventricular muscle fibres and on rabbit atrial fibres.

2 Berberine (3–30 μM) increased in a concentration-dependent manner, the action potential duration (APD) in canine Purkinje and ventricular muscle without affecting other parameters of the action potential.

3 The berberine-induced enlargement of the APD showed reverse use-dependence, so that the effect was greater at lower rates of stimulation.

4 Preparations perfused with berberine (30 μM) and driven at rates below 0.5 Hz exhibited early after depolarizations which persisted 3–4 h after washing.

5 The early afterdepolarizations were reversibly abolished by perfusion with lignocaine (3 μM) or by the increase in the rate of stimulation.

6 The effective refractory period (ERP) of Purkinje fibres was greatly increased by berberine (30 μM); however, the ratio ERP/APD was not significantly affected.

7 Berberine (10–100 μM) decreased in a concentration-dependent manner the spontaneous frequency of rabbit sinoatrial cells. The decrease in frequency was accompanied by a depression of the phase 4 depolarization, without significant changes in other parameters of the nodal action potential.

8 Atropine (2.5 μM) did not affect the bradycardic effect of berberine. On the other hand, berberine (30 μM) did not alter the chronotropic effect of isoprenaline.

9 Berberine (30 μM) also increased the duration of slow responses in K-depolarized rabbit atrial muscle fibres, other parameters being unaffected.

10 It is suggested that berberine exerts Class III antiarrhythimic and proarrhythmic actions in cardiac muscle of the dog *in vitro*.

Keywords: Berberine; canine myocardial fibres; rabbit atria; Class III antiarrhythmic agents; early afterdepolarization

Introduction

Berberine, an alkaloid found in numerous plants of the genera *Berberis* and *Coptis*, has been used as a medicinal agent for many centuries. It has been reported that berberine and some of its derivatives are potent α-adrenoceptor antagonists (Ko & Lim, 1980; Vizi *et al.*, 1986). Berberine has also shown positive inotropic activity in guinea-pig (Shaffer, 1985) and man (Marin Neto *et al.*, 1988). Antiarrhythmic (Krol *et al.*, 1982) and pro-arrhythmic (Marin Neto *et al.*, 1988) effects have also been found after i.v. administration of berberine in dogs and men. In the present investigation, by use of conventional microelectrode techniques, the electro-pharmacological effects of berberine were studied on segments of dog and rabbit hearts.

Methods

Adult mongrel dogs (10–15 kg) were anaesthetized with sodium thiobarbitone (50 mg kg^{-1}, i.v.) and their hearts were quickly removed through a left thoracotomy. The ventricles were opened and various pieces of muscle with attached false tendons were dissected and stored in cool oxygenated Tyrode solution. One piece was mounted in a 15 ml tissue chamber with a Sylgard-lined base. Rabbit atrial preparations were obtained and studied as described previously (Riccioppo Neto, 1982).

Tyrode solution aerated with 95% O_2 (pH 7.4) was kept at a temperature of 36 ± 0.5°C and flowed over the preparation at a rate of 12–15 ml min^{-1}. The Tyrode solution had the following composition (mM): NaCl 137, $CaCl_2$ 1.8, KCl 4 (for rabbit atrial tissue: KCl 5.4), $MgCl_2$ 0.45, $NaHCO_3$ 12, NaH_2PO_4 0.32 and glucose 5.5. In order to obtain the slow response an equimolar concentration of NaCl was substituted by KCl (25 mM) and isoprenaline (10^{-6} M) was added to the perfusion fluid.

Transmembrane potentials were recorded with conventional glass microelectrodes filled with 3 M KCl having resistances of 10–20 MΩ and displayed, via an input capacity neutralization preamplifier (Grass P16) on an oscilloscope (Tektronix 5113). The maximum rate of rise of the action potential was determined electronically with an OP-AMP (Analog 118 A). Oscilloscope traces were photographed on 35 mm film with a Nihon-Kohden PC-3A camera. For analysis of the sinoatrial rate, bipolar surface electrograms were recorded from the crista terminalis and the signal was simultaneously displayed on the oscilloscope.

Square wave pulses were obtained either from a Grass stimulator (S4-SIU4) or from a specially built stimulator so that an extra-stimulus (S2) could be delivered, with variable delay and amplitude, after the eight basic pulse (S1). S1 pulses (1.5 × threshold, 1 ms duration) and S2 pulses (3 × threshold, 1 ms duration) were delivered to the preparation

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through the same pair of Teflon coated silver wire electrodes. For studies of membrane refractoriness two recording microelectrodes were used. The effective refractory period (ERP) was measured as the shortest interval between S1 and S2 pulses needed to elicit an extrasystole that propagated to the distal microelectrode. The normal frequency of stimulation was 1.5 Hz.

Action potential characteristics were analysed by hand after enlargement of the film (7 ×) and the following parameters were measured: maximum diastolic potential (MDP), action potential amplitude (APA), maximum rate of rise of action potential (V_{max}), duration of the action potential from its peak to 50% (APD_{50}) and 90% (APD_{90}) repolarization, and sinoatrial rate.

Drugs used were: atropine sulphate (Merck); berberine chloride (Sigma); (−)-isoprenaline hydrochloride (Sigma); lignocaine hydrochloride (K & K). Unless otherwise stated, the results describe effects of drugs applied cumulatively during a single stable impalement.

Data are expressed as mean values ± s.e.mean and comparisons between two means were made by Student's paired *t* test. *P* values of less than 0.05 were considered to indicate significant differences.

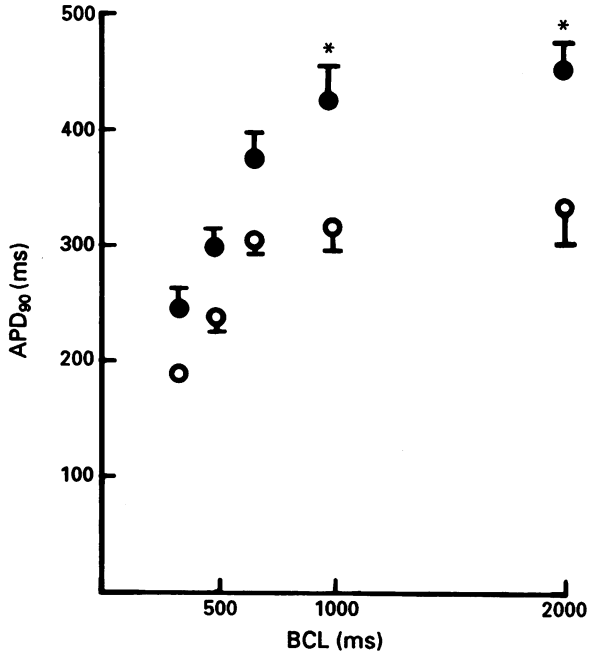

Figure 1 Action potential duration at 90% repolarization (APD_{90}) of Purkinje fibres ($n = 5$) driven at different rate before (○) and after berberine (30 μM) perfusion (●). BCL = basic cycle length. $^*P < 0.05$ compared with control at the same frequency.

Results

Effects on action potential parameters of dog Purkinje and ventricular muscle fibres

Berberine at concentrations between 1 and 30 μM prolonged the action potential duration (APD) of both Purkinje and ventricular muscle fibres in a concentration-dependent manner, without affecting the other action potential characteristics (Table 1). Concentrations up to 100 μM did not further increase the APD when tested upon Purkinje fibres. The prolongation of the APD induced by berberine was irreversible. The APD even increased slightly during washing in drug-free Tyrode solution and remained prolonged after 2 h of observation.

Effects of berberine on Purkinje fibres driven at different rates

Five Purkinje fibre preparations were initially stimulated at a frequency of 0.5 Hz and after consecutive intervals of 3 min to allow stabilization, the frequency of stimulation was increased to 1; 1.5; 2 and 3 Hz, respectively. Berberine (30 μM) was then perfused during 40 min and the stimulation at various frequencies repeated. As shown in Figure 1, the berberine-induced enlargement of the APD varied inversely with the rate of stimulation. Below 0.5 Hz four of the preparations studied showed oscillations of the membrane potential around −40 mV (Figure 2). These early afterdepolarizations (EADs) were reversibly blocked by the increase in the frequency of stimulation. In two preparations, the addition of a small concentration of lignocaine (3 μM) blocked reversibly the early afterdepolarizations (not shown). Berberine-induced EADs could be elicited during 3–4 h after washing off the drug, the only requirement being slow rate of stimulation.

Effects on membrane refractoriness

The effective refractory period (ERP) of Purkinje fibres treated with berberine (30 μM) was significantly increased in four experiments from 255 ± 12 ms (control) to 321 ± 15 ms ($P < 0.01$). However, since the total APD in the preparations was increased from 320 ± 10 ms (control) to 432 ± 9 ms (berberine-treated) the ratio ERP/APD decreased, although not significantly.

Table 1 Dose-related effects of berberine on the action potential parameters of the dog myocardium

	Berberine (μM)				
Purkinje fibres	0	3	10	30	100
APA (mV)	123 ± 2	122 ± 3	123 ± 3	121 ± 3	122 ± 3
MDP (mV)	90 ± 2	88 ± 3	88 ± 3	87 ± 3	88 ± 4
V_{max} (Vs^{-1})	690 ± 26	693 ± 23	700 ± 20	692 ± 28	686 ± 31
APD_{50} (ms)	226 ± 13	230 ± 16	267 ± 11	290 ± 17*	288 ± 16*
APD_{90} (ms)	275 ± 8	278 ± 6	316 ± 12*	408 ± 13**	410 ± 15**
Ventricular muscle					
APA (mV)	110 ± 3	111 ± 3	108 ± 2	109 ± 2	107 ± 3
MDP (mV)	85 ± 2	85 ± 2	87 ± 2	85 ± 3	84 ± 2
V_{max} (Vs^{-1})	440 ± 22	430 ± 29	425 ± 20	433 ± 24	428 ± 26
APD_{50} (ms)	118 ± 9	120 ± 8	135 ± 7	180 ± 13**	177 ± 10**
APD_{90} (ms)	178 ± 8	182 ± 10	199 ± 6	230 ± 10**	288 ± 9**

The data are presented as means ± s.e.mean of five preparations.
$^*P < 0.05$; $^{**}P < 0.01$, as compared with predrug control values.

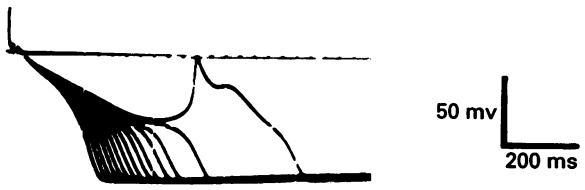

Figure 2 Superimposed action potentials of a berberine-treated (30 μM) Purkinje fibre stimulated at a decreasing frequency from 1.5 Hz (shortest action potential duration) to 0.1 Hz, in which the early afterdepolarization appeared.

Effects on the sinoatrial node of rabbits

In four preparations in which stable impalements could be maintained throughout, berberine was applied cumulatively (10–100 μM). There was a decrease in the rate of firing of nodal cells due to a depression of the phase 4 depolarization without major effects on other action potential parameters (Figure 3, inset).

In eight other preparations, atropine (2.5 μM) did not significantly reduce the negative chronotropic effect of berberine. On the other hand, the positive chronotropic effect of isoprenaline was not influenced by concomitant treatment with berberine (30 μM) (Figure 3).

Effects of berberine on the slow-response of rabbit atrium

Slow responses were obtained from four left atrium trabeculae driven at a rate of 0.3 Hz. After 40 min perfusion with berberine (30 μM) there was a consistent delay in the repolarization phase of the slow responses, the other parameters being unchanged. In these preparations APD_{50} and APD_{90} increased 55 ± 9% and 45 ± 6%, respectively, after berberine perfusion.

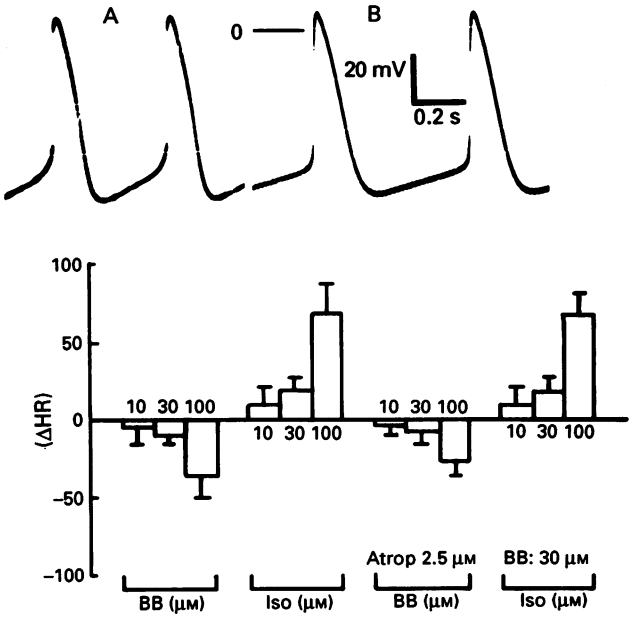

Figure 3 Variations in the spontaneous sinoatrial frequency (Δ HR) of isolated right atria of rabbits treated with berberine (BB; $n = 8$), isoprenaline (Iso; $n = 4$), berberine during atropine (Atrop; $n = 4$) and isoprenaline during berberine ($n = 4$). Inset: Effect of berberine (100 μM) on the action potentials of a S-A node cell; (A) control, (B) berberine, 30 min.

Discussion

Berberine induced a marked prolongation of the repolarization phase of the action potential on dog isolated ventricular myocardium without affecting the other action potential characteristics. The increase in the action potential duration (APD) exhibited reverse use-dependence (Hondeghem & Synders, 1990), a property common to drugs that have the greatest effect on prolonging repolarization at lower frequencies of stimulation like quinidine (Roden & Hoffman, 1985), sotalol (Strauss *et al.*, 1970) etc. Like these agents, berberine was also capable of inducing early after depolarizations (EADs) in preparations driven at slower rates. Manoeuvres such as lignocaine perfusion or an increase in the rate of stimulation were able to suppress reversibly the berberine-induced EADs as has been observed for EADs caused by quinidine (Valois & Sasyniuk, 1988; Davidenko *et al.*, 1989) and hypoxia plus acidosis (Rozanski & Witt, 1991).

Since no effect on V_{max} was detected in the range of concentrations used and the ratio ERP/APD was unaltered after berberine, the increase in the membrane refractoriness is probably the result of the increase in the APD. Local anaesthetic properties have been reported for berberine at higher concentrations, (Sabir & Bhide, 1971); no depression of the action potential amplitude of the rabbit vagus nerve was however found for berberine up to 0.5 mM (Riccioppo Neto, unpublished).

It seems reasonable, therefore, to attribute Class III action (Vaughan Williams, 1984) to berberine. Berberine has indeed been able to antagonize arrhythmias induced in dogs (Krol *et al.*, 1982; Kwiezycka *et al.*, 1983) and in rats (Ribeiro *et al.*, 1982), effects that could be accounted for by the drug-induced increase in refractoriness observed in the present experiments. Proarrhythmic effects such as the development of ventricular tachycardia with torsades de pointes morphology were also detected in men after i.v. infusion of berberine (Marin Neto *et al.*, 1988). There has been an increasing wealth of evidence suggesting that EAD is the basic cellular mechanism of torsades de pointes (El-Sherif *et al.*, 1989; Carlsson *et al.*, 1990) and the berberine-induced EADs observed *in vitro* could be one of the operative mechanisms responsible for the arrhythmia that ocurred in men.

The techniques employed in the present work do not permit a distinction of the underlying ionic mechanism(s) responsible for the lengthening of the APD caused by berberine. Although this can be achieved by a relative increase in inward over outward currents, it is believed that the great majority of Class III antiarrhythmic agents act primarily by blocking cardiac potassium channels which normally permeate outward currents (Carmeliet, 1985; Balser *et al.*, 1987).

In spontaneously beating right atria from rabbits, berberine caused a concentration-dependent decrease in the rate of diastolic depolarization. The consequent bradycardia was not blocked by atropine suggesting that muscarinic receptors were not involved. An atropine-resistant bradycardia after berberine has been previously described by Chun *et al.* (1979) in rats and by Shaffer (1985) in guinea-pig isolated right atria. A berberine-induced increase in spontaneous rate of isolated right atria from rabbits, bathed in Ringer-Locke solution at 30°C, is referred to by Sabir *et al.* (1978). Berberine has not been shown to possess adrenoceptor blocking action since it did not affect the chronotropic effect of isoprenaline in rabbit right atria. It has been recently reported that Class III antiarrhythmic agents like sotalol, bretylium and amiodarone at lower concentrations (10^{-6} M), prolonged significantly the cycle length of rabbit S-A node preparations without affecting other action potential characteristics (Satoh, 1991). In voltage-clamped preparations, despite some differences in potency and in the type of current affected, all three agents decrease the hyperpolarization-activated inward current, an effect that would result in a

decrease in the rate of spontaneous discharge. It remains an open question whether berberine could exert a similar action.

It is interesting to note that in guinea-pig atria, berberine caused positive inotropism not prevented by reserpinization or propranolol (Shaffer, 1985) and that in dogs with experimental heart failure berberine improved left ventricular function (Vik-Mo *et al.*, 1983). Selective lengthening of the APD may increase the transsarcolemmal calcium current, elevate the release of calcium from the sarcoplasmic reticulum and, as a consequence, increase the free calcium concentration close to the contractile elements (Reiter, 1988; Carlsson *et al.*, 1991). On the other hand it should be noted that an increase in tension development has been associated with either shortening or lengthening of the cardiac APD (Allen, 1977).

In isoprenaline-induced slow action potentials berberine produced an increase in APD. However, on slow-responses elicited by the application of histamine in potassium-depolarized guinea-pig papillary muscles, berberine (25 μM) increased significantly not only APD and the ERP, but also the action potential amplitude and V_{max} (Huang *et al.*, 1990). Since these two last effects were blocked by a very high concentration of propranolol (34 mM) it was argued that the inotropic effect of berberine could be the result of a β-receptor stimulating action (Huang *et al.*, 1990). It is, however, difficult to exclude nonspecific depressor effects of such an elevated concentration of propranolol upon the inotropic effect of berberine.

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