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Effects of cyproheptadine on electrophysiological properties of isolated cardiac muscle of dogs and rabbits

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1 The effects of cyproheptadine were studied on cardiac Purkinje and ventricular muscle fibres of the dog and on cells of the sinoatrial (SA) node region of rabbit hearts, by means of electrophysiological techniques.

2 Cyproheptadine (2–8 μM) decreased, in a dose-dependent manner, the plateau amplitude and the action potential duration to 50% repolarization of Purkinje and ventricular muscle cells. Higher concentrations also depressed the action potential amplitude, the overshoot and the maximum rate of rise of the upstroke. These effects were only partially reversed on washing.

2 A four fold increase in Ca concentration of the standard Tyrode solution antagonized the effects of cyproheptadine on the action potential characteristics.

4 The 'slow response' obtained in K-depolarized isoprenaline-treated fibres was blocked by cyproheptadine (4 μM).

5 Cyproheptadine (10 μM) depolarized and suppressed the automaticity of spontaneously beating Purkinje fibres.

6 The frequency of discharge of the SA node cells was slowed or abolished by cyproheptadine (2–4 μM). Atropine (2.6 μM) did not affect the negative chronotropic effect, whereas adrenaline (5 μM) reversed it.

7 It is suggested that cyproheptadine depresses the slow inward current in all types of myocardial fibres studied. Higher concentrations might also affect the fast inward sodium current system.

Introduction

Cyproheptadine, a widely known anti-5-hydroxytryptamine and anti-histamine agent (Casy, 1978), has been shown to depress the electrical excitability of nerves (Riccioppo Neto, 1979) and smooth muscle cells (Lowe, Matthews & Richardson, 1981). Whereas the local anaesthetic effect in nerve seems to be due to a blockade of sodium channels, the action of cyproheptadine in smooth muscle probably involves inhibition of the transmembrane calcium current through slow channels.

An antiarrhythmic effect of cyproheptadine was described, in whole animals, by Singh, Pendse & Bhandari (1975) and, more recently, by Helke, Quest & Gillis (1978). The antiarrhythmic properties of the antihistaminic agents in general are well known (Giotti & Zilletti, 1976).

The present study describes the effects of cyproheptadine on the membrane resting and action potentials of isolated cardiac Purkinje and ventricular muscle fibres of the dog and of the right atrium of the rabbit. Attempts have been made to correlate the observed effects with alterations in transmembrane current underlying excitability.

Methods

Adult mongrel dogs (15–20 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Their hearts were exposed through a lateral thoracotomy and rapidly removed. Both ventricles were opened and various pieces of muscle with false tendons attached were dissected and stored in cool oxygenated Tyrode solution. One piece was transferred to the Sylgard-lined bottom of a 15 ml tissue chamber. Right atrial preparations were obtained from rabbits and studied as described previously (Riccioppo Neto, 1982).

Tyrode solution aerated with 95% O_2 and 5% CO_2 (pH 7.4) flowed over the preparation at a rate of 5–6 ml min^{-1} and at a temperature of 36 ± 0.5°C.

Transmembrane potentials were recorded with conventional glass microelectrodes filled with 3M KCl having resistances of 10–20 MΩ and displayed, via an input capacity neutralization preamplifier (Grass P 16), on an oscilloscope (Tektronix 5112). The maximum rate of rise of the action potential was determined electronically using an OP-AMP (Analog Devices 118 A) and a RC circuit with time-constant of 50 μs. The output of the differentiator was linear with rates of potential change in the range of 50–1000 Vs^{-1}. Graphical differentiation was also made in some experiments by measuring the slope of a straight line drawn by eye through the steepest part of the upstroke. Oscilloscope traces were photographed on 35 mm film with a camera (Nihon-Kohden PC-3A). Changes in resting potential were simultaneously recorded on a servo-driven potentiometric pen recorder.

Square wave pulses were obtained from a Grass stimulator (S4-SIU4) and delivered through a pair of Teflon coated silver wire electrodes. Preparations were driven at a cycle length of 800 ms using pulses 0.5 ms in duration and twice the threshold.

In three experiments with right papillary muscles, two microelectrodes were inserted: one in an attached Purkinje fibre, another (5–10 mm beyond) on the adjacent muscle.

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), overshoot of action potential, maximum rate of rise of action potential (V_{max}), duration of action potential from its peak to 50% (ADP_{50}) and 90% (APD_{90}) repolarization and sinoatrial rate. The plateau amplitude (PA) was measured at 20 ms from the upstroke for Purkinje fibres, a time which nearly coincides with the peak slow inward current in voltage clamp conditions (Vassalle, 1979) and at 30 ms for muscle fibres (Beeler & Reuter, 1977; Munakata, Dominic & Surawicz, 1982). Unless otherwise stated, the results describe effects of drugs applied at cumulative concentrations during a single stable impalement.

Data are expressed as mean values ± s.e.mean. Statistical analysis was performed by Student's *t* test for the difference of means and *P* values of less than 0.05 were considered to indicate significant differences.

The Tyrode solution had the following composition (mM): NaCl 137, $CaCl_2$ 1.8, KCl 4, $MgCl_2$ 0.45, $NaHCO_3$ 12, NaH_2PO_4 0.32 and glucose 5.5. For studies of automaticity, KCl was 2 mM for Purkinje fibres and 5.4 mM for rabbit atrial preparations. In order to obtain the 'slow response' an equimolar concentration of NaCl was substituted by KCl (25 mM); isoprenaline (1 μM) was added to the perfusion fluid and the preparations were driven at a rate of 0.1 Hz.

Drugs used: (−)-adrenaline (Sigma); atropine sulphate (Merck); cyproheptadine hydrochloride (gift from Merck); diphenhydramine hydrochloride (Sigma); (−)-isoprenaline hydrochloride (Sigma).

Results

Effects on action potential characteristics of dog Purkinje fibres

The effects of cyproheptadine (2–16 μM) usually appeared within 10–15 min after the beginning of the perfusion and stabilized in about 30–40 min. As shown in Figures 1a and 2a, the most remarkable effects were a decrease in APD_{50} concomitant with a reduction in PA. Higher concentrations (16 μM or above) also decreased APA, the overshoot, APD_{90}, V_{max} and induced secondary action potentials occurring eventually during the course of the repolarization. Three preparations treated with 32 μM cyp-

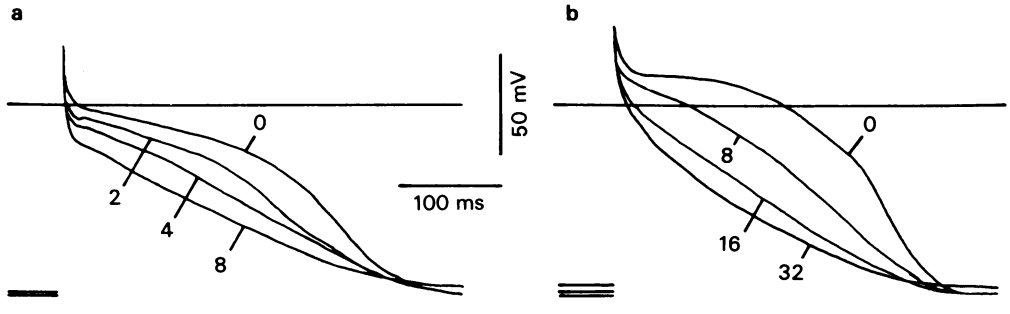

Figure 1 Superimposed action potentials of canine Purkinje fibres treated with cyproheptadine (30 min for each concentration). (a) Standard Tyrode solution; (b) another preparation bathed in 7.2 mM Ca^{2+} Tyrode solution. The number attached to each trace represents the concentration of cyproheptadine (μM). Calibrations: vertical trace: 50 mV; horizontal trace: 100 ms.

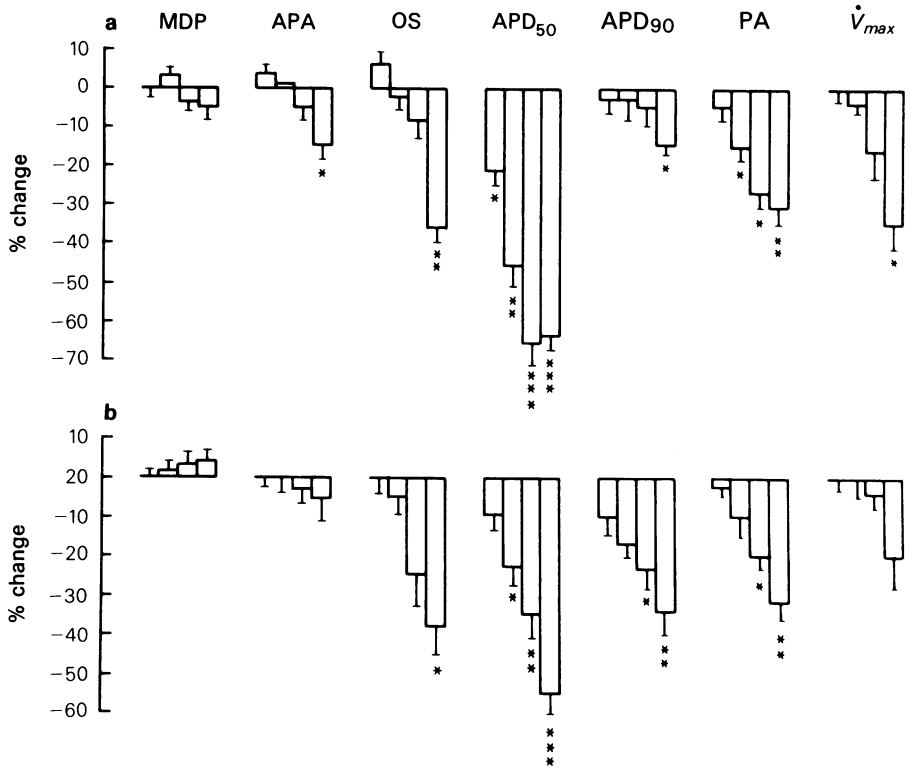

Figure 2 Percentage changes induced by increasing concentrations of cyproheptadine (2, 4, 8 and 16 μM respectively for each parameter measured) on the transmembrane voltage characteristics of Purkinje ($n = 4$; a) and right papillary muscle fibres ($n = 4$; b) of the dog. MDP: Maximum diastolic potential; APA: action potential amplitude; APD_{50} and APD_{90}: action potential duration at 50% and 90% repolarization, respectively; PA: plateau amplitude; V_{max}: maximum rate of rise of the action potential. $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$. Student's *t* test for the difference of means from control.

roheptadine showed complete inexcitability after 20 min of perfusion. During the drug perfusion a negative inotropism was clearly seen through microscopic observation. Washing of the preparations for about 1 h, after sequential exposure to different concentrations of the drug, did not produce full recovery. In five preliminary experiments in which only single concentrations of cyproheptadine were applied, complete reversibility was found for only 2 and 4 μM. A fast and complete recovery was, however, obtained throughout the entire range of concentrations if the drug-free perfusion fluid contained an increased (4×) calcium concentration.

A comparison was made with another antihistaminic agent, diphenhydramine (20 μM), perfused for 30 min. In three preparations the effects appeared in the first 10 min of perfusion and consisted of a fall in APA (10.33 ± 3.93%), overshoot (37.33 ± 7.72%), APD_{50} (14.00 ± 4.1%) and V_{max} (15.66 ± 3.02%). The other parameters were practically unaffected. The effects of diphenhydramine were rapidly reversed upon washing. In two experiments diphenhydramine (20 μM) was perfused for 30 min after a four fold increase in the extracellular calcium concentration. The only detectable effect was a 18.02 ± 3.2% reduction in the APD_{50} of the treated fibres.

Effects on automaticity in dog Purkinje fibres

The effects were examined in five preparations exposed for 30 min, to a single concentration of cyproheptadine (10 μM). As illustrated in Figure 3, there was a depolarization and a gradual suppression of the spike, but no significant slowing in the frequency of discharge. As already found for the driven fibres, a four fold increase in extracellular calcium concentration, accelerates the time for recovery after cyproheptadine treatment.

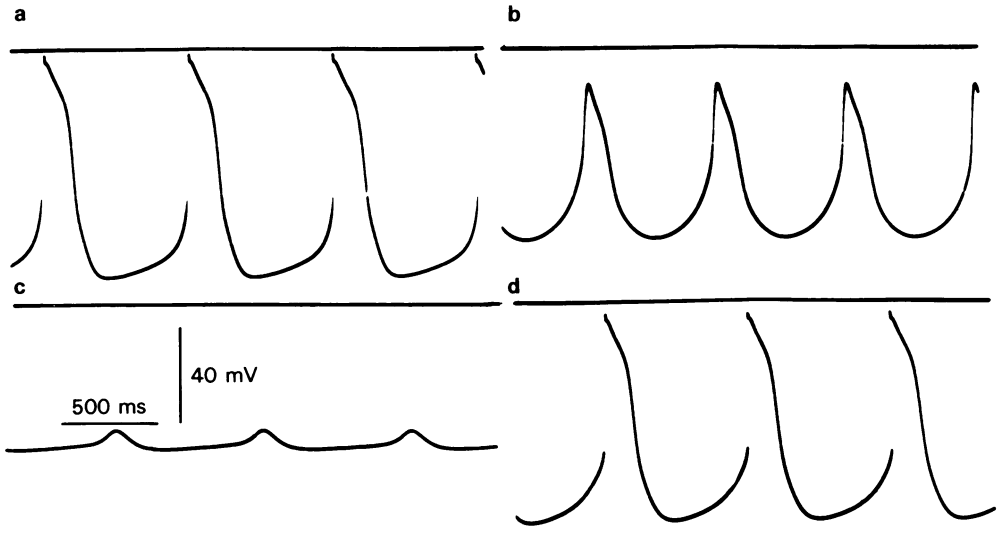

Figure 3 Effects of cyproheptadine (10 μM) on a spontaneously beating Purkinje fibre. (a) Control; (b) and (c) 20 and 25 min, respectively, after the addition of cyproheptadine to the Tyrode solution; (d) after washing of the preparation for 30 min with drug-free solution. Vertical and horizontal calibrations respectively: 40 mV and 500 ms.

Effects of an increase in the extracellular calcium concentration

In four preparations the effects of cyproheptadine were tested after an increase in the extracellular calcium concentration from 1.8 mM to 7.2 mM, by addition of $CaCl_2$ to the standard Tyrode solution. An equilibration period of 15 min in high calcium solution was allowed. A typical record of the results obtained is shown in Figure 1b. Higher concentrations of cyproheptadine (8–32 μM) were needed in order to induce effects similar to those obtained in preparations perfused with Tyrode containing normal calcium concentration. The effects of cyproheptadine on APA, overshoot and V_{max} were also antagonized by the increased calcium concentration.

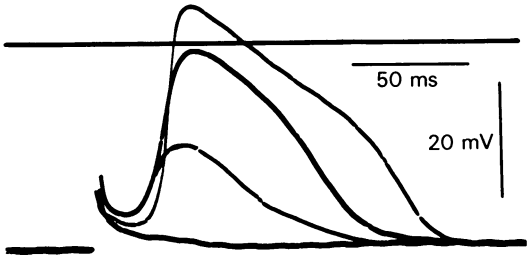

Figure 4 Superimposed 'slow responses' in K^+-depolarized isoprenaline-treated Purkinje fibres, before (larger trace) and during the application of cyproheptadine (4 μM). The gradually decreasing responses were obtained at 15, 20, 25 and 30 min during the drug perfusion. Vertical and horizontal calibrations respectively: 20 mV and 50 ms.

Effects on the ventricular muscle action potential

These effects were studied in seven right papillary muscles. In three of them a simultaneous record of the transmembrane potentials of the attached Purkinje fibre was made. Cyproheptadine (2–16 μM) induced similar effects on both types of fibres, as shown in Figure 2b. Results obtained from multiple impalements of right papillary muscle cells also indicated that a four fold increase in the extracellular calcium concentration antagonized the effects of cyproheptadine on the action potential characteristics of the ventricular muscle.

Effects on the 'slow response'

Cyproheptadine (4 μM) abolished reversibly the 'slow response' obtained from Purkinje fibres in four experiments (Figure 4). The same concentration, however, was without effect in three preparations bathed in Tyrode containing an increased calcium concentration (7.2 mM). A greater concentration of cyproheptadine (16 μM) was needed to block the 'slow response' of these fibres.

Effects on the sinoatrial node of rabbits

Cyproheptadine (2 and 4 μM) was applied cumulatively in three preparations in which a stable impale-

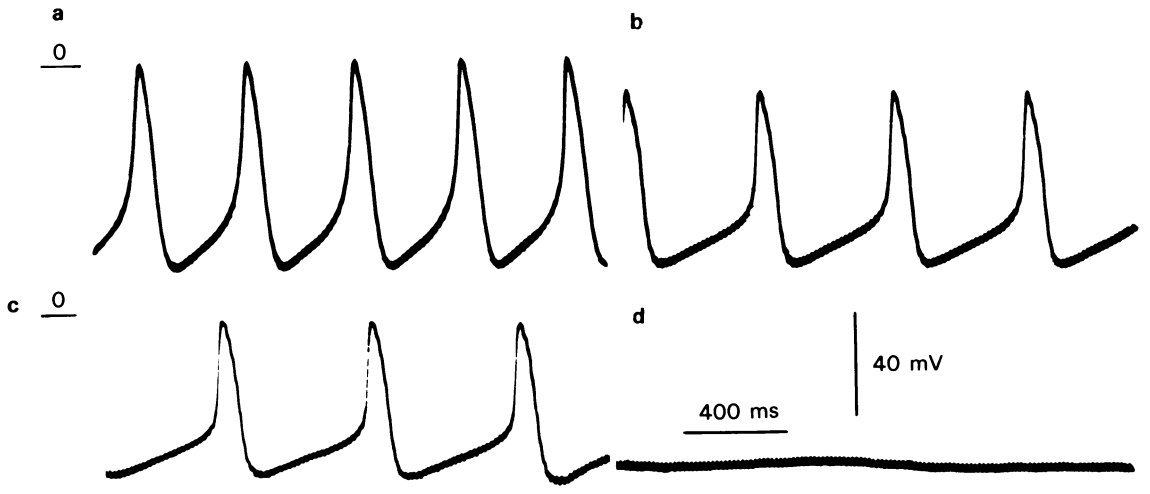

Figure 5 Effects of cyproheptadine on the electrical activity of a S-A node cell of the rabbit. (a) Control; (b) after 40 min in pressure of 2 μM cyproheptadine; (c) 15 min in presence of 4 μM cyproheptadine; (d) 5 min after (c). The reference line is labelled 0. Vertical calibration: 40 mV; horizontal calibration: 400 ms.

ment was maintained throughout (Figure 5). The smaller concentration slowly reduced the APA (16.00 ± 6.08%), the rate of diastolic depolarization and the sinoatrial rate (15.33 ± 2.7%), whereas 4 μM depolarized the cells (18.02 ± 4.33% fall in MDP) and further decreased APA towards complete block (Figure 5 c,d).

Multiple impalements were made in five other preparations. Atropine (2.6 μM) did not affect the negative chronotropic effect of cyproheptadine (4 μM) in two of these preparations and adrenaline (5 μM) greatly increased the diminished frequency of three preparations previously treated by cyproheptadine (2 μM).

Discussion

The most significant effects of cyproheptadine on canine cardiac Purkinje and ventricular muscle fibres were a simultaneous decrease in APD_{50} and in the plateau amplitude of the action potentials. Both effects were readily antagonized by a four fold increase in the extracellular calcium concentration.

This would suggest a cyproheptadine-induced reduction of inward calcium current through the slow channel. In fact, there is evidence showing that cyproheptadine can block calcium-mediated physiological actions in various preparations. Cyproheptadine inhibits the release of insulin and glucagon from the perfused rat pancreas (Joost, Beckmann, Holze, Lenzen, Poser & Hasselblatt, 1976), inhibits the insulin secretion in islets of Langerhans isolated from rats (Richardson, 1976; Donatsch, Lowe, Richardson & Taylor, 1980), reduces the spontaneous contraction of the rat uterus (Sadovsky, Dora, Pfeifer, Polishuk, Rachamimoff & Sulman, 1973) and suppresses mechanical and electrical activity in guinea-pig taenia coli (Lowe *et al.*, 1981). These effects are antagonized in high-calcium media. Cyproheptadine also decreases the ^{45}Ca uptake into strips of guinea-pig taenia coli (Lowe *et al.*, 1981). On Langendorff-perfused guinea-pig heart, cyproheptadine, after an initial brief increase in the contractile force, induced a dose-dependent negative inotropism concomitant with a decrease in the perfusion pressure and a slight negative chronotropism. These effects are prevented by an increase in the extracellular calcium concentration (Antonio & Riccioppo Neto, unpublished).

The effects of cyproheptadine on the action potential characteristics of Purkinje fibres are similar to those described for verapamil and methoxyverapamil (D-600) (Kohlhardt, Bauer, Krause & Fleckenstein, 1972; Cranefield, Aronson & Wit, 1974; Rosen, Ilvento, Gelband & Merker, 1974). On the other hand, diphenhydramine, another antihistaminic chosen for comparison did not reduce the plateau amplitude of the action potential and its effect on APD_{50} was not antagonized in high-calcium medium. At low concentrations diphenhydramine, like other antihistaminics, probably does not affect the slow inward current system (Johnson, 1956; Johnson & McKinnon, 1957).

Consistent with the hypothesis of blockade of the slow inward current by cyproheptadine are the depressor effects on the 'slow response' of Purkinje

fibres and on sinoatrial discharges of the rabbit heart. A slow inward current underlies the slow diastolic depolarization and the upstroke of the action potential of sinoatrial cells (Brown & Di Francesco, 1980; Brown, 1982) and agents which inhibit the slow inward current, such as verapamil, also depress sinus node discharge (Wit & Cranefield, 1974). The negative chronotropic effect induced by cyproheptadine is probably not due to a release of acetylcholine from nerve terminals or to an activation of muscarinic receptors, since it was not blocked by atropine. On the other hand adrenaline, which has been shown to increase the slow inward current of sinoatrial cells (Brown, Di Francesco & Noble, 1979; Noma, Kotake & Irisawa, 1980), overcame the effects of cyproheptadine.

At higher concentrations cyproheptadine might also be exerting depressor effects on the fast inward sodium current system since it also decreased APA, V_{max} and overshoot of Purkinje and muscle fibres. A local anaesthetic effect of cyproheptadine has already been described (Riccioppo Neto, 1979). An antagonistic effect of calcium upon the effects of cyproheptadine on the rapid depolarization phase was also found in the present work and would be expected (Weidmann, 1955; Hille, 1977).

It is difficult to say how much of the suppression of the spontaneous activity in Purkinje fibres was due to the depolarization. It should be added that higher concentrations of verapamil induced similar phenomena in canine Purkinje fibres (Rosen *et al.*, 1974).

With the present findings it remains uncertain whether and to what extent a cyproheptadine-induced increase in the potassium conductance may contribute to the reduction in plateau amplitude and APD_{50} of Purkinje and muscle fibres. However, contrary to what we have observed, an increase in gK would have produced hyperpolarization and perhaps an acceleration in the total repolarization time.

Voltage clamp studies are needed in order to confirm some of our findings and to uncover other possible actions of cyproheptadine on the ionic permeabilities of the membranes.

Antiarrhythmic actions could be attributed to cyproheptadine on the basis of its electrophysiological effects. However, at a concentration of only 4 μM, the drug causes sinoatrial arrest and a large effect on APD_{50} and PA which suggests a strong negative inotropic effect. These observations probably indicate that antiarrhythmic actions might be associated with highly undesirable effects.

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