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Aspects of the cardiovascular pharmacology of exaprolol

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This study confirms that exaprolol is a potent β-adrenoceptor antagonist, having a pA_2 value of 9·8 for the inhibition of the inotropic and chronotropic responses of guinea-pig isolated atrial preparations to isoprenaline. In the anaesthetized cat, exaprolol blocks both the myocardial stimulatory and vasodilating effects of isoprenaline, suggesting that it is a non-selective antagonist at β-adrenoceptors. Exaprolol also has direct electrophysiological effects on cardiac (Purkinje) tissue, reducing the rate of rise of phase 0 of the action potential. This direct action together with its marked blockade of β-adrenoceptors may explain the drug's ability to markedly suppress the ischaemic ventricular arrhythmias that follow coronary artery occlusion in anaesthetized rats.

Exaprolol (MG 8823), a cyclohexylphenol, with β-blocking and local anaesthetic properties (Carissimi et al 1976), has been recently shown to reduce epicardial ST-segment elevation when administered after the induction of myocardial ischaemia in anaesthetized cats (Parratt & Udvary 1980, 1983). The fact that, in those studies, the administration of exaprolol resulted in β-adrenoceptor blockade without significant myocardial depression and reduced at least one index of myocardial ischaemia suggested that this compound might have potential in the early therapy of acute myocardial infarction (Parratt & Udvary 1983). The main purpose of the present studies was to examine the myocardial effects of exaprolol in more detail and to investigate a possible protective effect against early ischaemia-induced ventricular arrhythmias.

METHODS

Isolated guinea-pig atrial and papillary muscle

Guinea-pigs of either sex (500–800 g) were killed by a blow to the head and the hearts removed. The left atria and papillary muscles were impaled on thin silver wire electrodes and stimulated at respective frequencies of 3 and 1 Hz, whilst the right atria beat spontaneously. All tissues were mounted in organ baths containing physiological salt solution (composition in mM litre^{-1}) NaCl 114, KCl 4·7, $MgCl_2$ 1·2, $CaCl_2$ 2·5, NaH_2PO_4 1·3, $NaHCO_3$ 2·5, glucose 5·6) bubbled with 95% O_2, 5% CO_2 and maintained at a temperature of 32 °C. Isometric tension was recorded after the tissues had been placed under a resting tension of 500 mg.

After a 30 min equilibration period, cumulative dose response curves to the positive inotropic and chronotropic action of isoprenaline were determined. The tissues were subsequently equilibrated with the lowest concentration of exaprolol (3×10^{-9} M) for 35 min and dose response curves to isoprenaline obtained in the presence of the antagonist. This procedure was repeated for two further concentrations of the antagonist (9 and 27×10^{-9} M). For each concentration of antagonist, dose-ratio values were obtained and Arunkashana & Schild plots constructed from which pA_2 values were determined (Arunkashana & Schild 1959).

In a further three experiments the direct effects of exaprolol on the tension developed by papillary muscle were examined by administration of three successive concentrations of the antagonist for 30 min periods.

Anaesthetized cat preparation

Eight cats (either sex, 1·8–2·2 kg) were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} i.p). Body (midoesophageal) core temperature was maintained between 36·5 and 38 °C. After tracheotomy the animals were artificially respired with room air delivered by a Palmer positive pressure ventilation pump (stroke volume 45–75 ml; rate 20 min^{-1}). Systemic arterial and right atrial pressures were recorded using capacitance transducers. Left ventricular pressure was measured via a stiff catheter inserted by way of the right carotid artery or by direct left ventricular puncture. The left ventricular pressure pulse was continuously differentiated to provide an index of myocardial contractility and end-diastolic pressure (LVEDP) was measured by cutting off the intraventricular pressure pulse above 20 mmHg. Left ventricular pressure, LVEDP, left ventricular dP/dt,

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systemic arterial pressure, right atrial pressure and the electro-cardiogram (leads I or II) were recorded on an eight-channel, ink-jet writing recorder (Mingograph 81). Cardiac output was determined by thermodilution, the details of which are described by Parratt (1974).

Isoprenaline (0·25 μg kg^{-1} min^{-1}) and noradrenaline (1·0 μg kg^{-1} min^{-1}) were infused intravenously for 5 min before, and at different times after, the slow intravenous administration of exaprolol (1·0 and 2·0 mg kg^{-1}). The haemodynamic parameters were measured before, during and immediately after each drug administration.

Coronary artery ligation in the anaesthetized rat

Male Sprague-Dawley rats (250–350 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1} i.p.) and artificially ventilated (stroke volume, 2 ml/100 g; 54 strokes min^{-1}). Carotid arterial blood pressure and a standard lead I or II electrocardiogram (ecg) were recorded using a mingograph 81 ink-jet recorder (Elema Schönander). A femoral vein was cannulated for drug administration. Rectal temperature was maintained at approximately 38 °C. The chest was opened between the fourth and fifth ribs approximately 2 mm to the left of the sternum. After the pericardium had been opened the heart was exteriorized and a 6/0 silk suture was placed under the left coronary artery as described by Selye et al (1960). The heart was re-positioned in the thoracic cavity, and the blood pressure and the ecg allowed to stabilize for 15min. After this time, either saline or exaprolol (0·5 and 2·0 mg kg^{-1}) were given i.v. and the ligature tied 15 min later. The severity of the arrhythmias was assessed by noting mortality, the incidence and duration of ventricular fibrillation (VF), and ventricular tachycardia (VT, defined as any run of seven or more consecutive ventricular extrasystoles), and by counting the total number of ventricular extrasystoles in the 0–30 min postligation period.

Electrophysiological studies

Sheep Purkinje fibre tissue pinned to the silastic base of the recording chamber was superfused at a rate of 5 ml min^{-1} with a physiological salt solution of composition (mM): NaCl, 125; $NaHCO_3$, 25; NaH_2PO_4, 1·2; $MgCl_2$, 0·5; KCl, 5·4; $CaCL_2$, 1·8; glucose 5·5 equilibrated with 95% O_2, 5% CO_2 and with temperature maintained at 35 ± 1 °C.

The tissue was stimulated at a frequency of 1 Hz by rectangular pulses 1 ms in duration and twice threshold voltage, delivered through a bipolar silver electrode. Through the same electrode a second pulse 1 ms in duration and three times threshold voltage, could be applied after every sixth driving stimulus for the determination of the absolute refractory period. Transmembrane action potentials were recorded using conventional microelectrode techniques. The parameters measured were: resting membrane potential (RMP); action potential height; the maximum rate of depolarization of phase zero (MRD), which was determined by an electronic differentiating circuit, the action potential duration at 50 and 90% repolarization levels (APD50 and APD90) and the absolute refractory period.

A stock solution of exaprolol, dissolved in 0·9% NaCl (saline), was added to reservoirs of gassed physiological salt solution to obtain final concentrations of 0·3, 1 and 3 mg $litre^{-1}$ (0·9, 3 and 9 × 10^{-8} M). In each experiment action potentials were recorded before and 1 h after the cumulative addition of each concentration of antagonist.

Statistics

The statistical significance of differences between means was calculated by a Student's *t*-test. A chi-square test was used to analyse the statistical significance of differences in the incidences of arrhythmias between groups. Mean values ± s.e.m. are quoted.

RESULTS

Isolated cardiac muscle

The pA_2 values calculated for the antagonism of isoprenaline on atrial preparations by exaprolol were 9·83 ± 0·02 and 9·82 ±0·02 for the positive inotropic and chronotropic responses respectively. A similar value (9·8) was obtained in papillary muscle preparations. Although for the inotropic responses, the antagonism was competitive, in some of the spontaneously beating preparations the maximum increase in heart rate obtained with isoprenaline was not achieved in the presence of the antagonist, suggesting a possible direct effect of exaprolol on the sino-atrial node.

In 2 out of 3 papillary muscles and 2 out of 5 paced atria, exaprolol initially increased developed tension and in no preparations did the drug, at β-blocking concentrations, depress isolated cardiac muscle.

Haemodynamic studies in the cat

The haemodynamic effects of injection of exaprolol in 5 cats are shown in Table 1. In a dose of 1 mg kg^{-1}, the drug caused bradycardia, and reductions in arterial pressure, LV dP/dt_{max} and in cardiac output.

Table 1. Cardiovascular effects* of exaprolol (1·0 mg kg^{-1}) given intravenously to anaesthetized cats.

	Systemic arterial pressure (mmHg) LVP		(mmHg)		LV dP/dt$_{max}$	Heart rate	Cardiac output	Stroke volume
	systolic	diastolic	LVP	LVEDP	(mmHg s^{-1})	(beats min^{-1})	(ml min^{-1})	(ml min^{-1})
Control	139 ± 10	105 ± 7	133 ± 11	3·3 ± 0·8	3110 ± 460	208 ± 4	287 ± 24	1·37 ± 0·11
Exaprolol (1 mg kg^{-1})	−23 ± 6	−24 ± 6	−29 ± 8	−0·8 ± 0·6	−1080 ± 440	−44 ± 8	−74 ± 22	−0·07 ± 0·1

* Values given are the changes (means ± s.e.m. of 5 observations) obtained 5 min after the slow i.v. injection of exaprolol.

The reduction in LV dP/dt$_{max}$ probably resulted in part from changes in preload and after load and in part from inhibition of sympathetic drive. A larger dose of exaprolol (2 mg kg^{-1}), given 2·25 h later, did not cause a more pronounced fall in either LV dP/dt$_{max}$ or in cardiac output; in fact the haemodynamic effects were slightly less than with the lower dose. The effect of isoprenaline (0·25 μg kg^{-1} min^{-1}) on diastolic blood pressure, heart rate and LV dp/dt$_{max}$ were markedly attenuated by exaprolol (1 mg kg^{-1}). Before administration of the drug, this dose of isoprenaline decreased diastolic pressure by 13 ± 4 mmHg and increased heart rate by 61 ± 4 beats min^{-1}, LV dP/dt$_{max}$ by 1800 ± 250 mmHg s^{-1} and cardiac output by 79 ± 32 ml min^{-1}. Thirty min after exaprolol administration isoprenaline *increased* diastolic blood pressure (by 6 ± 4 mmHg) whilst the increases in heart rate, LV dP/dt$_{max}$ and cardiac output were much reduced (20 ± 7 beats min^{-1}, 900 ± 300 mmHg s^{-1} and 30 ± 5 ml min^{-1}). When the isoprenaline infusions were repeated 1 h later (i.e. 1·5 h after exaprolol administration) there was full recovery of β_1-mediated cardiac responses (e.g. increase in heart rate of 54 ± beats min^{-1} and of LV dP/dt$_{max}$ of 1660 ± 320 mmHg s^{-1}) although isoprenaline-induced vasodilatation was still antagonized (*increases* in diastolic blood pressure cf. 4 ± 3 mmHg).

Noradrenaline-induced pressure responses were not modified by exaprolol (1 or 2 mg kg^{-1}). For example noradrenaline (1 μg kg^{-1} min^{-1}) increased diastolic blood pressure by 28 ± 6 mmHg (from 102 ± 8 mmHg) before exaprolol and by 25 ± 12 mmHg afterwards; noradrenaline-induced increases in LV dP/dt$_{max}$ (resulting from both increased cardiac contractility and increases in after load) were reduced by the drug (1190 ± 250 mmHg s^{-1} before and 680 ± 280 s^{-1} after exaprolol administration).

Coronary artery ligation-induced arrhythmias

In all of the control rats, coronary artery ligation resulted in marked ventricular ectopic activity and ventricular tachycardia during the first 30 min; 62% of the animals fibrillated. Since spontaneous reversion to sinus rhythm often occurs in this model, mortality was much lower than this (17%; Table 2). Exaprolol (0·5 and 2·0 mg kg^{-1}) markedly reduced the number of ectopic beats as well as the incidence and duration of VT and fibrillation. None of the animals given a dose of 2·0 mg kg^{-1} fibrillated (Table 2). Two animals given the drug (one in each dose group) died after ligation from A–V block leading to asystole.

Both doses of exaprolol caused a marked bradycardia in anaesthetized rats; heart rates before ligation were 451 ± 10, 335 ± 16 and 341 ± 11 in the

Table 2. The effect of exaprolol on mortality and on the occurrence and degree of arrhythmic activity resulting from acute coronary artery ligation in anaesthetized rats.

Treatment	n	Mortality	Ventricular ectopic beats (number)	Ventricular tachycardia (s)	Ventricular fibrillation (s)
Saline	35	6/35 (17%)	1262 ± 153 (100%)	73 ± 12 (100%)	62 ± 14 (62%)
Exaprolol (0·5 mg kg^{-1})	6	1/6 (17%)	563 ± 191 (100%)	52 ± 16 (80%)	48 (20%)
Exaprolol (2·0 mg kg^{-1})	7	1/7 (14%)	301 ± 91* (100%)	16 ± 6* (83%)	0* (0%)

n = Number of animals. Percentage incidence of arrhythmias is shown in parenthesis. *$P < 0·05$.

saline, exaprolol (0·5 mg kg^{-1}) and exaprolol (2·0 mg kg^{-1}) treated groups respectively. The effects of the two doses of antagonist were similar, suggesting that maximum β-blockade had been achieved with the lower dose. Upon ligation, no significant changes in heart rate occurred in any of the groups.

There was also an initial transient fall in mean arterial pressure on injection of both doses of exaprolol. By 5 min after the injection, however, pressure had returned to the pre-injection level of 98 ± 4 mmHg. There was no significant difference in the pressures of any of the groups, at the time the ligature was tied. Coronary artery ligation led, in all groups, to a slight systemic hypotension which was maintained in the rats administered the higher dose of exaprolol and at 30 min post-ligation blood pressure in this group of animals was less (69 ± 10 mmHg) than in the control group (84 ± 4 mmHg).

Electrophysiological studies

The effects of exaprolol on sheep Purkinje fibre action potentials are shown in Table 3. The most pronounced effects, evident in the lowest concentration of 0·3 mg litre^{-1} were a reduction in the maximum rate of depolarization (MRD) of phase 0 and a shortening of the duration of the action potential. The absolute refractory period (ARP) was also shortened by exaprolol (1 mg litre^{-1}) from 311 ± 12 to 210 ± 10 ms (n = 4); this was related to the shortening of the action potential duration since the ratio of ARP/APD90 was similar in the presence of the drug (1·20) to the control situation (1·13). The resting membrane potential was unchanged except at the highest concentration of exaprolol (3 mg litre^{-1}). Recovery from these effects was complete 2 h after superfusion with normal Tyrode solution.

DISCUSSION

These results confirm that exaprolol is a potent β-adrenoceptor antagonist. In-vitro, the pA_2 value for inhibition of the inotropic and chronotropic responses to isoprenaline (9·8) is slightly greater than that of propranolol in this preparation (8·7) (Blinks 1967). There is some indication from the isolated studies that the drug may possess intrinsic sympathomimetic activity since it had a transient positive inotropic action in some of the preparations. However, spontaneous heart rate was not increased, and in fact the maximum chronotropic response to isoprenaline was not achieved in the presence of the antagonist, suggesting a possible direct depressant action on the sino-atrial node.

The compound is not selective for β_1-adrenoceptors since in-vivo it blocked both the myocardial stimulatory and vasodilating actions of isoprenaline. At a dose of 1 mg kg^{-1} in the cat, β-blockade lasted for about 1·5 h, a more prolonged action than is observed with, for example, an equivalent dose of alprenolol (Parratt & Wadsworth 1970). As with alprenolol (Parratt & Wadsworth 1970), the recovery from blockade of β_2-mediated responses was slower than that of myocardial β_1-adrenoceptors. Exaprolol had no effect on vascular α receptors at least in doses up to 2 mg kg^{-1}. Unlike propranolol (Parratt 1969) exaprolol did not potentiate the noradrenaline-induced pressor response, implying that it does not influence the uptake of noradrenaline.

Besides its potent β-blocking properties, exaprolol has direct electrophysiological effects on cardiac tissue. On sheep Purkinje tissue, it markedly reduced the maximum rate of depolarization of phase 0 of the action potential which, in absence of changes in resting membrane potential, indicates a

Table 3. The effect of exaprolol (0·3, 1 and 3 mg litre^{-1}) on sheep Purkinje fibre action potential characteristics.

Dose of exaprolol mg litre^{-1}	No. of cells	R.M.P. mV	A.P. height mV	MRD V/s	APD50 ms	APD90 ms
Control	40	86·8 ± 0·6	120·9 ± 0·8	466 ± 13	244·2 ± 7·5	352·6 ± 6·7
0·3	40	85·7 ± 0·4 (−1)	115·0 ± 1·0* (−5)	409 ± 12* (−12)	174·8 ± 5·8* (−28)	290·4 ± 5·3* (−18)
1	40	85·2 ± 0·7 (−2)	110·1 ± 1·2* (−9)	326 ± 13* (−30)	130·9 ± 2·5* (−46)	252·8 ± 3·5 (−28)
3	26	81·4 ± 0·6* (−6)	83·6 ± 1·6* (−31)	135 ± 9* (−71)	93·9 ± 1·9 (−62)	220·0 ± 5·3* (−38)

The % change from control values are given in parentheses. *$P < 0·01$.

drug-induced reduction in the fast inward sodium current. This action may also underly the shortening of the action potential duration that was observed since it has been shown that a tetrodotoxin-sensitive sodium current contributes to the maintenance of the plateau in this tissue (Coraboeuf et al 1979). Other blockers such as propranolol have similarly been shown to block sodium channels (Morales-Aquilerá & Vaughan Williams 1965) and to shorten action potential duration in Purkinje fibres (Harrison et al 1973). This shortening of the action potential duration by exaprolol was accompanied by a proportional reduction in the absolute refractory period, an action which may be considered to be pro-arrhythmic. However, it has also been postulated that, for example during ischaemia, such an action may prevent the formation of re-entry circuits and hence contribute to antiarrhythmic activity (Rosen et al 1975). Indeed exaprolol did prevent coronary artery ligation induced arrhythmias in the anaesthetized rat (Table 2). At 2 mg kg^{-1}, it completely abolished the occurrence of ventricular fibrillation and markedly reduced the number of extrasystoles. In this model exaprolol is more effective than propranolol and sodium channel blocking drugs such as ORG 6001, lignocaine and quinidine (Kane et al 1979). The combination of marked β-adrenoceptor blockade and the ability to reduce the rate of rise of phase 0 of the cardiac action potential may underly its efficacy in protecting against ischaemia-induced arrhythmias.

In summary, exaprolol is a potent non-selective antagonist at β-adrenoceptors in the cardiovascular system. It also blocks sodium entry into cardiac Purkinje cells via the fast channels, an action which may contribute to its marked effectiveness against ischaemia-induced arrhythmias.

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