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Effect of acute and chronic treatment with practolol on cardiovascular responses in the pithed rat

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Chronic administration of practolol to the pithed rat produced a reduction in the pressor responses to electrical stimulation of the spinal cord and potentiation of pressor responses to high doses of (—)-noradrenaline compared to control animals. Acute administration of practolol caused an increase in the pressor responses to both electrical stimulation and high doses of noradrenaline. Heart rate responses to both forms of stimulation were less than control values after both acute and chronic dosage with practolol. It is possible that practolol reduces the release of noradrenaline at the sympathetic nerve ending after chronic administration.

The mechanism by which β-adrenoceptor blocking agents reduce blood pressure in hypertensive disease has not been elucidated. Various mechanisms have been suggested as to their mode of action in this condition (Prichard & Gillam, 1966, 1969; Shanks, 1967; Frohlich, Tarazi & others, 1968; Eliash & Weinstock, 1971; Michelakis & McAllister, 1972). It has also been suggested that the antihypertensive action of β-adrenoceptor blocking agents may be related to a reduction in the reflex activity of the sympathetic nervous system (Ester & Nestel, 1973). None of these explanations has as yet been entirely satisfactory.

The adrenergic-neuron blocking effect of propranolol is now well established (Day, Owen & Warren, 1968; Mylecharane & Raper, 1970; Barrett & Nunn, 1970, *in vitro* and Eliash & Weinstock, 1971, *in vivo*) but has never been demonstrated successfully with practolol (Barrett & Nunn, 1970). An attempt has, therefore, been made to determine whether acute or chronic administration of practolol to the pithed rat would alter the changes in heart and blood pressure produced by (—)-noradrenaline or electrical stimulation of the sympathetic outflow according to Gillespie & Muir (1967).

METHODS

Control. Eighteen female Wistar rats, 250 g, were injected intraperitoneally twice daily with 1·5 ml of saline for six weeks. Each rat was then injected with atropine (1 mg kg^{-1}, i.p.) anaesthetized with ether and the trachea cannulated. A stainless steel pithing rod was then passed down the spinal cord and left *in situ*, the indifferent electrode being placed under the skin of the femoral region (Gillespie & Muir, 1967). The animals were respired artificially with a Palmer respiratory pump. Blood pressure was recorded via the carotid artery by means of a Bell and Howell 4–327–L221 blood pressure transducer connected to a Devices M2 pen recorder. The pulse pressure was differentiated and used as an input signal to a cardiotachometer connected to the pen recorder. The jugular vein was cannulated for administration of drugs.

Noradrenaline was administered in random order in doses of 10, 50, 100 and 500 ng. Each dose was given three times and the responses of blood pressure and heart rate recorded. When the series of administration of noradrenaline was complete, the rats were given 5 mg kg^{-1} of pancuronium bromide which caused complete neuromuscular blockade, and no change in resting blood pressure or heart rate was recorded after its injection. This was followed by stimulation via the pithing rod-electrode. Stimulation was achieved by a Scientific and Research Instruments Stimulator (S.R.I. Ltd. England). The parameters were: square wave pulse 20 Hz for 10 s at 20, 30, 40 or 50 V. Each stimulus was applied on three occasions at 5 min intervals in random order. All measurements of rises in heart rate and blood pressure were incremental from pre-stimulation levels. The data were analysed using Student's *t* test.

Chronic. Eight female Wistar rats weighing 250 g at the start of the experiment were injected twice daily with practolol (13 mg kg^{-1} i.p.) dissolved in saline (1·5 ml) for six weeks. The pH of the practolol solution was 5·8. Responses of blood pressure and heart rate were recorded after injection of noradrenaline and electrical stimulation of the spinal cord, as described.

Acute. Ten female Wistar rats of 250 g were injected with noradrenaline intravenously followed by electrical stimulation of the cord as described, after administration of practolol (13 mg kg^{-1}, i.v.).

Drugs used were (—)-noradrenaline bitartrate (Sigma), pancuronium bromide (Organon), practolol (ICI) and atropine sulphate (Koch-Light). All doses were expressed in terms of the free base except atropine sulphate and pancuronium bromide.

RESULTS

Control. There was a dose-dependent rise in heart rate and blood pressure in the control animals after noradrenaline or electrical stimulation. Typical responses to electrical stimulation and noradrenaline are shown in Figs 1a and 2a. All responses are tabulated in Tables 1–4.

Chronic. The resting blood pressure (systolic S and diastolic D) and heart rate of the pithed rats are shown in Table 1. After chronic treatment with practolol the

Table 1. *Initial blood pressure and heart rate of control pithed rats and after acute and chronic treatment with practolol.*

		Blood pressure (mm Hg)	Heart rate (min^{-1})
Control	S	56·4 ± 4·7	330 ± 8·7
n = 18	D	41·3 ± 3·5	
Acute	S	72·1 ± 4·4	414 ± 10·1
n = 10	D	52·4 ± 3·7	
Chronic	S	72·9 ± 4·6	334·3 ± 26·9
n = 8	D	58·9 ± 4·1	

S = Systolic B.P. D = Diastolic B.P.

Blood pressure (systolic): Controls less than acute, $P < 0·05$. Controls less than chronic, $P < 0·05$; Acute no significant difference to chronic.

Blood pressure (diastolic): Controls less than acute, $P < 0·05$; Controls less than chronic, $P < 0·01$; acute no significant difference to chronic.

Heart rate: Controls less than acute, $P < 0·001$. Controls not significantly different to chronic; acute greater than chronic, $P < 0·01$.

Table 2. *Incremental rise of mean arterial blood pressure and heart rate in control pithed rats following noradrenaline administration and electrical stimulation of spinal cord.*

Dose of noradrenaline (ng)	Rise in b.p. (mm Hg)	Rise in heart rate
10	23·5 ± 2·6	19·9 ± 2·2
50	41·0 ± 3·2	44·5 ± 2·2
100	51·6 ± 3·5	59·9 ± 2·5
500	78·2 ± 4·8	92·2 ± 2·2
Voltage		
20	74·8 ± 6·2	38·1 ± 4·0
30	102·7 ± 7·0	54·4 ± 4·6
40	116·3 ± 7·0	61·4 ± 4·7
50	129·4 ± 7·0	65·8 ± 4·7

Figures are means ± s.e. of 18 control animals.

Table 3. *Incremental rise of mean arterial blood pressure and heart rate in pithed rats previously treated for 6 weeks with practolol and following noradrenaline administration and electrical stimulation of the spinal cord.*

Dose of noradrenaline (ng)	Rise in b.p. (mm Hg)	Rise in heart rate
10	18·7 ± 1·9	0
50	40·7 ± 1·9	0·4 ± 0·3
100	56·2 ± 2·3	3·7 ± 0·8
500	95·1 ± 2·6	14·8 ± 2·9
Voltage		
20	57·9 ± 10·2	6·1 ± 1·1
30	73·3 ± 10·4	10·7 ± 0·3
40	90·2 ± 10·0	12·6 ± 0·5
50	112·3 ± 10·7	15·9 ± 0·5

Figures are means ± s.e. of 8 chronically treated animals.

Table 4. *Incremental rise of mean arterial blood pressure and heart rate in pithed rats treated acutely with practolol and following noradrenaline administration and electrical stimulation of the spinal cord.*

Dose of noradrenaline (ng)	Rise in b.p. (mm Hg)	Rise in heart rate
10	19·7 ± 1·1	0
50	46·0 ± 1·7	0·4 ± 0·2
100	60·2 ± 1·9	0·9 ± 0·3
500	111·8 ± 2·6	4·4 ± 0·8
Voltage		
20	123·6 ± 4·8	5·8 ± 0·4
30	147·2 ± 5·5	9·7 ± 0·7
40	157·5 ± 6·4	12·7 ± 1·1
50	163·9 ± 5·8	15·9 ± 1·3

Figures are means and s.e. of 10 acutely treated animals.

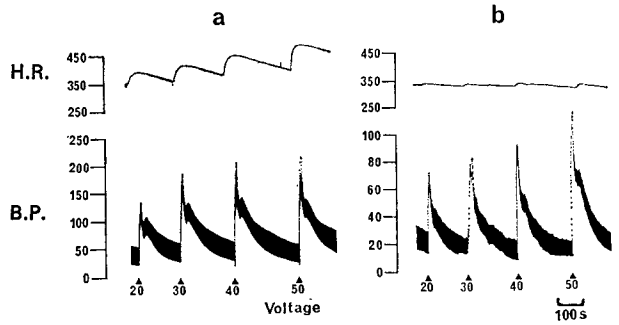

FIG. 1. Responses of blood pressure and heart rate to electrical stimulation of the spinal cord in the pithed rat: (a) Control responses. (b) Responses after 6 weeks treatment with practolol. Scale for B.P. = mm of Hg. Scale for H.R. = beats min^{-1}.

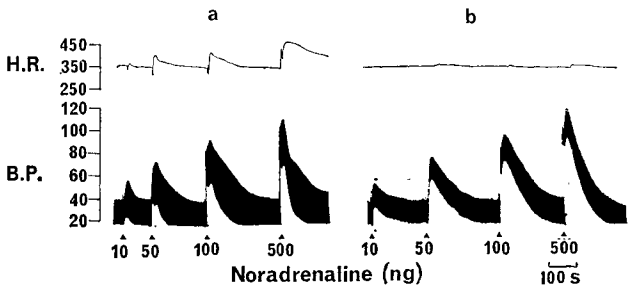

FIG. 2. Responses of blood pressure and heart rate to (−)-noradrenaline in the pithed rat. (a) Control responses. (b) Responses after 6 weeks treatment with practolol. Scale for B.P. = mm of Hg. Scale for H.R. = beats min^{-1}.

systolic and diastolic pressures were significantly greater than the control values (S, $P < 0{\cdot}05$; D, $P < 0{\cdot}01$) and the heart rates were not significantly different.

After twice daily dosage of practolol for six weeks there was a statistically significant ($P < 0{\cdot}01$) potentiation of the pressor response to 500 ng noradrenaline. Following electrical stimulation in these chronically treated animals the incremental rise in blood pressure was less than in the control animals. The deficit was statistically significant for the 30 and 40 V stimulations ($P < 0{\cdot}05$). The tachycardia produced by noradrenaline and electrical stimulation was less after chronic treatment with practolol than in the control animals. This difference was statistically significant at all dose and voltage levels $P < 0{\cdot}001$). Typical responses to both types of stimulation for this series of experiments are shown in Figs 1b and 2b.

Acute. After acute treatment with practolol the initial systolic and diastolic pressures in the pithed animals were significantly higher than control values ($P < 0{\cdot}05$ for S & D), and the heart rate also significantly greater ($P < 0{\cdot}001$). The initial values for acute and chronically treated rats for blood pressure were not significantly different from each other but heart rate showed a significantly higher value in the acutely treated animals ($P < 0{\cdot}01$).

Blood pressure responses to noradrenaline after acute administration of practolol showed significant potentiation at the 100 and 500 ng dose levels ($P < 0{\cdot}05$ and $0{\cdot}001$ respectively). Practolol produced a marked potentiation of the rise in blood pressure at all voltage levels of electrical stimulation ($P < 0{\cdot}001$). The pressor responses to

electrical stimulation at all voltage levels in the acutely treated animals were significantly greater than those in the chronically treated group ($P < 0{\cdot}001$).

Both forms of stimulation (electrical and noradrenaline) caused tachycardia. After acute or chronic administration of practolol the resulting increase in heart rate was significantly less than before treatment ($P < 0{\cdot}001$). There was no difference in this respect between acutely and chronically treated animals except for the response to 500 ng noradrenaline where the tachycardia was greater in chronically than acutely treated animals ($P < 0{\cdot}001$).

DISCUSSION

The responses to noradrenaline and stimulation of the sympathetic cord outflow of pithed rats, chronically and acutely treated with practolol, have been compared to the response of the control group which had previously been treated with saline. The pithed animal was unable to tolerate many injections of noradrenaline or electrical stimuli and as a result it was not possible to use each animal as its own control. The dose of practolol used was chosen on a weight basis as being approximately equivalent to the administration of 1 g of practolol to a 75 kg man. The usual oral dose of practolol to man is 100–400 mg twice daily, however, doses of up to 3000 mg day^{-1} have been used for the control of hypertension (Prichard, Boakes & Day, 1971). The results show that tachycardia after noradrenaline and electrical stimulation was diminished in rats treated acutely or chronically with practolol. This result was to be expected in view of the fact that practolol is a cardioselective β blocking drug (Dunlop & Shanks, 1968). Since the animals were pithed the reflex bradycardia normally produced by noradrenaline was abolished.

Although no difference in systolic and diastolic pressures were noted between the two practolol-treated groups, these values were significantly higher than the corresponding controls. However, because the heart rate after a single injection was significantly greater than that measured in rats given practolol chronically this may signify a higher peripheral resistance in the latter group.

It is difficult to explain the potentiation of pressor responses by noradrenaline after acute and chronic administration of practolol. Meier (1972) has already described a similar potentiation of the pressor response to noradrenaline in cats, after practolol administration. Meier considers that this effect of practolol could be due to a slight inhibition of catecholamine uptake (Foo, Jowett & Stafford, 1968).

The most striking feature about these results is that the pressor responses after electrical stimulation in the rats treated with practolol for six weeks were less than the pressor responses in the animals given practolol as a single intravenous dose, or the control animals. The largest difference in the pressor responses due to electrical stimulation occurred between the groups of animals treated acutely and chronically with practolol. The mechanism underlying these results is not yet known.

The reduction in blood pressure responses produced by electrical stimulation in the chronically treated group is interesting since it would appear that this is either due to reduced release of noradrenaline at the adrenergic nerve terminals or to reduced production of noradrenaline in the adrenergic neuron with consequent reduction in release. Barrett & Nunn (1970) failed to show an adrenergic neuron blocking effect with practolol in the isolated rat vas, and Nayler & Chang (1972) failed to show any effect of practolol on the release of noradrenaline from dog heart after stimulation of the stellate ganglion. Both of these investigations were done as acute experiments,

and it is conceivable that some time, i.e. weeks, is needed before this effect is demonstrable with practolol. Recent work has shown that 6 months oral therapy with practolol in hypertensive subjects reduced urinary noradrenaline output in response to head up tilt. As already stated it is suggested by this recent work that the antihypertensive action of β-adrenoceptor blockers may be related to a reduction in the reflex activity of the sympathetic nervous system (Ester & Nestel, 1973). The other explanation would be that practolol interferes with noradrenaline storage in the adrenergic neuron. It has been shown (Masurkiewicz-Kwilecki & Romagnoli, 1970) that chronic treatment of rats with propranolol, significantly reduced catecholamine stores in the myocardium. Although these workers found no change in the average systolic blood pressure after 9 weeks of treatment, their animals were not challenged with electrical stimulation to see whether release of endogenous noradrenaline was affected. Practolol was not, however, included in their study.

By reducing the reuptake of noradrenaline by the adrenergic neuron over a number of weeks stores might become depleted. This is unlikely since there would be a reduction in the negative feedback mechanism which regulates noradrenaline stores in the adrenergic neuron; unless this mechanism is also blocked by chronic treatment with practolol. A reduction in the noradrenaline released from adrenergic neurons may also explain the diminished pressor responses to electrical stimulation in the chronically treated animals. The evidence contradicting this mechanism of action of practolol and other β-blocking drugs in hypertension is that they have been found to produce no postural or orthostatic hypotension in man (Prichard & others, 1971), unlike guanethidine or other adrenergic neuron blocking drugs. The results would indicate that an action at the adrenergic neuron may be involved in the mechanism of the hypotensive properties of practolol and possibly of other β-blocking drugs.

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