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Cardioregulatory properties of indoramin in the rat

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The antihypertensive action of the competitive α-adrenoceptor antagonist indoramin is not accompanied by reflex tachycardia in animals or man. The possibility that the established local anaesthetic property of indoramin is involved in its cardioinhibitory action has been investigated. Indoramin evoked a dose-dependent bradycardia in anaesthetized/pithed rats. The decrease in heart rate was slightly greater than that evoked in anaesthetized intact animals suggesting that indoramin had a direct action on the heart. Reflex tachycardia was simulated in pithed rats by increasing the frequency of cardiac nerve stimulation from 0·3 to 1 Hz. Indoramin and the local anaesthetic agents, lignocaine and procaine, reduced the positive chronotropic response without markedly altering the basal rate. The response curves were parallel. In contrast, phentolamine decreased the positive chronotropic response, but only at high doses which were associated with a marked decrease in the basal rate. Thymoxamine and prazosin had no significant effects on the chronotropic response. These experiments suggest that the cardioregulatory action of indoramin is attributable to its local anaesthetic property and this action further distinguishes it from the other α-adrenoceptor antagonists tested.

The decrease in blood pressure evoked by a number of peripherally acting antihypertensive agents is accompanied by tachycardia. This is due, at least in part, to an increase in sympathetic nervous tone following activation of the baroreceptor reflex. The antihypertensive action of the competitive α-adrenoceptor antagonist indoramin is not accompanied by reflex tachycardia in either animals or man (Baum et al 1973; Carballo et al 1974) and in anaesthetized animals indoramin evokes hypotension and bradycardia (Alps et al 1972a; Baum et al 1973). In vitro studies suggest that indoramin has a direct action on the myocardium. Alps et al (1972b) showed that concentrations of indoramin $>10^{-6}$ M reduced the rate of contraction of the rabbit isolated heart and Coltart et al (1971) demonstrated a decrease in the rate of depolarization of canine ventricular muscle strips with concentrations ($2{\cdot}6 \times 10^{-7}$ M) which are known to occur in plasma after oral hypotensive doses in man.

It has been suggested that the actions of indoramin on the cardiac conduction system may result from its potent local anaesthetic membrane stabilizing properties (Alps et al 1970a, 1971).

The purpose of the present study was twofold. Firstly, experiments have been made to confirm that the bradycardia evoked by indoramin in anaesthetized animals is the result of a direct cardiac action. Secondly, evidence has been sought to test the hypothesis that, in the presence of indoramin, the heart is unable to respond to a rapid increase in sympathetic nerve activity owing to a decreased electrical responsiveness of the myocardium. The effects of indoramin have been compared with those of standard local anaesthetics and α-adrenoceptor antagonists.

MATERIALS AND METHODS

Drugs

Indoramin mesylate, thymoxamine hydrochloride (Warner), phentolamine mesylate (Ciba), prazosin hydrochloride (Pfizer), lignocaine hydrochloride (Ward Blenkinsop) and procaine hydrochloride (Sigma) were used. Drugs were dissolved in either distilled water or 0·9% NaCl (saline) and administered cumulatively every 15 min, doubling from the first dose. Drug doses were calculated as base.

Anaesthetized and anaesthetized/pithed rats

Two groups of female Charles River rats (250–300 g) were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.). Blood pressure and heart rate were recorded from a carotid artery (Bell and Howell pressure transducer, Devices M19 polygraph). The trachea was intubated and one group of rats was allowed to breathe spontaneously. The other group was pithed and respiration maintained artificially (Palmer pump, 1 ml/100 g, 60 strokes

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min^{-1}). Rectal temperature was maintained at 37 °C (Palmer thermal blanket). Indoramin (0·8–25·6 mg kg^{-1}) was administered to both groups via a cannula in the jugular vein and blood pressure and heart rate recorded at 15 min after each dose.

Sympathetic nerve stimulation in pithed rats

Groups of four female Charles River rats (200–400 g) were anaesthetized with 5% halothane in oxygen and pithed. Respiration and rectal temperature were maintained as described above. The left femoral vein was cannulated for drug administration. Arterial pressure was measured from the left femoral artery (Bell and Howell pressure transducer) and integrated heart rate was derived using the pressure wave to trigger a tachograph. Both cardiovascular variables were displayed on a Devices M19 polygraph. The sympathetic nerves supplying the heart were stimulated selectively by two electrodes integral with the pithing rod. The electrodes were positioned as described in an earlier communication (Algate & Waterfall 1978). Tubocurarine (1 mg kg^{-1} i.v.) was administered to prevent skeletal muscle contraction during electrical stimulation.

The cardiac sympathetic outflow was stimulated continuously (0·3 Hz, 0·5 ms, 25 V). The positive chronotropic response following an increase in stimulation frequency to 1 Hz was measured 5 min before and 10 min after drug administration. The higher stimulation frequency was maintained until the maximum increase in heart rate was achieved (usually within 1·5 min) following which the frequency was reduced to 0·3 Hz. Each phase of 1 Hz stimulation was followed by a 5 min recovery period. The frequencies of 0·3 and 1 Hz were chosen as being representative of low and moderate levels of sympathetic tone (Iriuchijima 1973).

Data analysis

A two way analysis of variance was used to test whether indoramin significantly decreased the spontaneous heart rates of anaesthetized and anaesthetized/pithed rats. Differences between the two groups were compared by regression analysis.

For the stimulated rats a two way analysis of variance was used to compare the effects of different dose levels of each drug on the evoked tachycardia and on the basal heart rate. Regression analysis was used to compare each drug with its vehicle.

RESULTS

Indoramin in anaesthetized and anaesthetized/pithed rats

The mean heart rates of the groups of anaesthetized and anaesthetized/pithed rats were 385 ± 20 (n = 6) and 299 ± 11 (n = 8) beats min^{-1} respectively. The effects of indoramin on these rats were expressed as a percentage of the pre-dose value in order to compensate for the decrease in heart rate evoked by pithing. Indoramin (0·8–25·6 mg kg^{-1}) evoked a significant dose-dependent bradycardia in both groups of rats (Fig. 1). Equations for best fit straight lines were $y = -0{\cdot}0069x + 4{\cdot}4748$ (anaesthetized rats) and $y = -0{\cdot}0168x + 4{\cdot}5239$ (anaesthetized/pithed rats) where $y = \log_e$ mean response and x = dose. The linear correlation for both groups was highly significant ($r = -0{\cdot}99$, $P = 0{\cdot}002$ and $r = -0{\cdot}98$, $P = 0{\cdot}0005$ respectively). The slope for the anaesthetized/pithed group was significantly greater than that for the anaesthetized group.

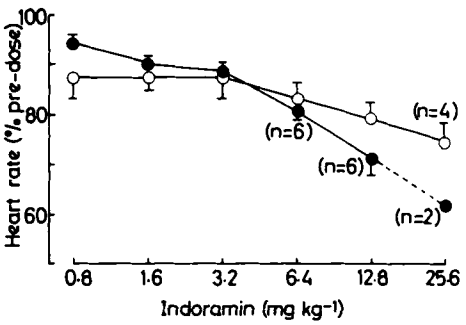

FIG. 1. The effect of cumulative doses of indoramin on the spontaneous heart rates of anaesthetized (○) (n = 6) and anaesthetized/pithed (●) (n = 8) rats. Readings taken 15 min after administration are expressed as a percentage of the pre-dose value so that the effects of pithing can be disregarded. Indoramin evoked a dose dependent bradycardia in both groups of rats which was significant at all doses (two way analysis of variance). Ordinate: heart rate (% pre-dose). Abscissa: dose of indoramin (mg kg^{-1} i.v.). Bars represent s.e.m. Number of surviving animals shown in parentheses.

The pre-dose diastolic pressures of the two groups of rats were 124 ± 4 mmHg (anaesthetized) and 40 ± 2 mmHg (anaesthetized/pithed). Indoramin dose dependently decreased blood pressure in the anaesthetised rats (−37 mmHg at 0·8 mg kg^{-1}; −63 mmHg at 25·6 mg kg^{-1}), but evoked no further decrease in the anaesthetized/pithed group.

Local anaesthetics and α-adrenoceptor antagonists on the electrically evoked tachycardia in pithed rats

In untreated animals an increase in frequency of sympathetic nerve stimulation from 0·3 to 1 Hz increased heart rate from 302 ± 8 beats min^{-1} to 354 ± 9 beats min^{-1} (n = 32).

Neither distilled water nor saline produced any consistent changes in the positive chronotropic response to sympathetic nerve stimulation. Ligno-

caine (0·01–1·3 mg kg^{-1}), procaine (0·1–12·8 mg kg^{-1}) and indoramin (0·2–25·6 mg kg^{-1}) evoked a dose-related inhibition of the positive chronotropic response which was significant at doses $\geq$0·02, $\geq$0·2 and $\geq$0·4 mg kg^{-1} respectively (Fig. 2). Phentolamine weakly inhibited the response at doses $\geq$3·2 mg kg^{-1}, the effect being statistically significant only at doses $\geq$12·8 mg kg^{-1}. Prazosin (0·01–2·6 mg kg^{-1}) and thymoxamine (0·1–1·6 mg kg^{-1}) had no significant effects on the positive chronotropic response. The difference between the slope of the regression of drug response on dose level and the slope of the regression of vehicle response on dose level was statistically significant for lignocaine, procaine, indoramin and phentolamine (P <0·001).

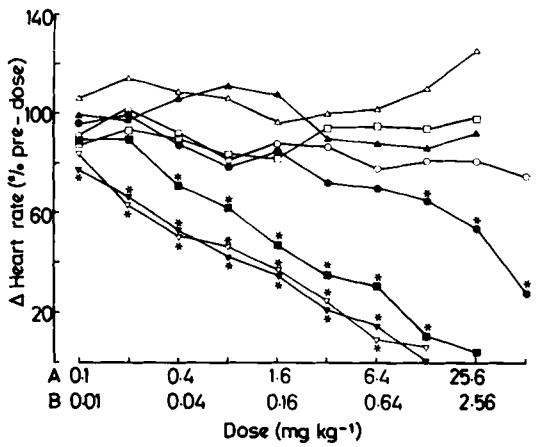

FIG. 2. The effect of α-adrenoceptor antagonists and local anaesthetics on the positive chronotropic responses of the heart to an increase in frequency of cardiac nerve stimulation (0·3 Hz to 1 Hz) in groups of 4 pithed rats. Ordinate: change in heart rate (% pre-dose increase). Abscissa: drug dose (mg kg^{-1}); scale A: indoramin (■), phentolamine (●), thymoxamine (▲), procaine (▼); scale B: prazosin (△), lignocaine (▽). Saline (□) and distilled water (○) vehicles were given in equivalent volumes. *P < 0·05 (two way analysis of variance).

Basal heart rate in the present experiments was defined as the rate evoked by continuous sympathetic stimulation at the lower frequency (0·3 Hz). The effects on basal heart rate of vehicles and drugs are shown in Fig. 3. Indoramin ($\geq$0·4 mg kg^{-1}) and distilled water vehicle evoked small, but statistically significant decreases in basal heart rate. However, regression analysis showed that the effect of indoramin was not significantly different from that of the vehicle alone. Phentolamine evoked a marked dose-related decrease in basal rate whereas thymoxamine slightly increased basal rate. Prazosin, lignocaine, procaine and saline had no significant effects on basal heart rate. Comparison of the slopes of regression lines showed that phentolamine was the only drug tested to have a significantly greater effect on basal heart rate than vehicle alone (P <0·001).

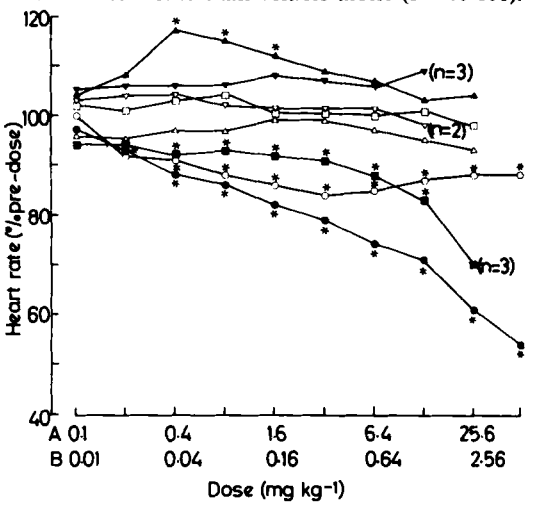

FIG. 3. The effect of α-adrenoceptor antagonists and local anaesthetics on the basal heart rate evoked by cardiac nerve stimulation at 0·3 Hz in groups of 4 pithed rats. Ordinate: heart rate (% pre-dose). Abscissa: drug dose (mg kg^{-1}); scale A: indoramin (■), phentolamine (●), thymoxamine (▲), procaine (▼); scale B: prazosin (△), lignocaine (▽). Saline (□) and distilled water (○) vehicles were given in equivalent volumes. Numbers of surviving animals shown in parentheses. * P < 0·05 (two way analysis of variance).

DISCUSSION

Since the dose-dependent decrease in heart rate evoked by indoramin in anaesthetized/pithed rats was even greater than that recorded in rats with an intact central nervous system, it is likely that the cardioinhibitory effect was due to a direct action on the myocardium. The membrane stabilizing action reported by Coltart et al (1971) in canine isolated ventricular strips provides a reasonable explanation for this effect. The bradycardia in both groups of rats was evoked at doses within the hypotensive range for the anaesthetized rats. The fact that there were no additional effects on blood pressure in the anaesthetized/pithed rats is consistent with the primarily sympatholytic mode of action of indoramin proposed by Alps et al (1970b).

The cardiac effects of indoramin have generally been attributed to its potent local anaesthetic properties (Alps et al 1970a, 1971). However, the effects of indoramin on tachycardias evoked in

experimental animals have not previously been compared with those of recognized local anaesthetic agents. Since the generation of reflex tachycardia poses difficulties, notably in regard to its reproducibility, the nervous sympathetic tone to the heart was maintained artificially by continuously stimulating the cardiac nerves at a frequency comparable with that of basal rates in the conscious animal (Iriuchijima 1973). Reflex tachycardia was mimicked by raising stimulation frequency to 1 Hz. This method provided a means of comparing the effects of drugs on the response of the heart to a constant, reproducible change in sympathetic nerve activity.

The curves generated for the dose-dependent inhibition of the positive chronotropic response evoked by the local anaesthetic agents procaine and lignocaine were parallel. Lignocaine was approximately 10 times as potent as procaine. The curve for indoramin was also parallel with those of the local anaesthetic agents suggesting a similar mechanism of action. Dose-dependent inhibition of the chronotropic response occurred at levels which were hypotensive in the anaesthetized rats and which have been shown to be hypotensive in other studies (Alps et al 1972a; Baum et al 1973; Oates et al 1977). Procaine and lignocaine were approximately 3 and 30 times more potent as inhibitors of the chronotropic response than indoramin. Previous experiments have shown that the potency of indoramin as a local anaesthetic is similar to that of lignocaine and procaine (Alps et al 1971). It seems likely therefore that the cardioregulatory action of indoramin in these experiments is due to a direct local anaesthetic membrane stabilizing action although other factors such as its lack of antagonist activity at cardiac presynaptic α-adrenoceptor sites (Algate & Waterfall 1978) may also be involved.

The results for indoramin are in contrast to those obtained for the other α-adrenoceptor antagonists. Phentolamine weakly inhibited the positive chronotropic response, a significant effect being detected only at doses in excess of the established hypotensive dose range in rats (Oates et al 1977). Thymoxamine and prazosin had no significant effect on the positive chronotropic response even at high doses. In this context, a number of reports associate tachycardia with the antihypertensive action of prazosin in animals (Commarato et al 1978) and man (Bailey 1977; Hua et al 1978).

The basal heart rate in the pithed rat was maintained by low frequency (0·3 Hz) electrical stimulation. Of the compounds tested, only phentolamine had a marked effect on the basal rate and was the only drug found to be consistently more inhibitory than vehicle alone. Langer et al (1977) concluded that the negative chronotropic response to phentolamine observed in rat isolated atria was a veratramine-like action. This property may explain the action of phentolamine on both the stimulated tachycardia and the basal rate. Under the present experimental conditions potentiation of basal heart rate due to presynaptic α-adrenoceptor antagonism was not observed. The potentiation of the basal rate by thymoxamine could be related to the weak noradrenaline uptake inhibitory properties of this compound (Drew et al 1978).

In conclusion, at doses within the hypotensive range, indoramin behaves similarly to standard local anaesthetics but differs from other α-adrenoceptor antagonists in that it can inhibit tachycardia evoked by an increase in frequency of sympathetic nerve stimulation. It is likely therefore that the absence of reflex tachycardia accompanying the antihypertensive action of indoramin in animals and man is the result of a local anaesthetic action.

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