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Catecholamine uptake blockade in anaesthetized dogs: influence on cardiovascular responses

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The effects of differential and combined catecholamine uptake antagonism on cardiovascular responses of anaesthetized dogs to isoprenaline, noradrenaline, and electrical stimulation of the left ansa subclavia nerve have been studied. $Uptake_1$ inhibition by cocaine HCl (5 mg kg^{-1} and 1 mg kg^{-1} every 45 min) enhanced responses to noradrenaline (0·1 to 2·0 $\mu g\ kg^{-1}$ i.v.) and sympathetic nerve stimulation (1 to 20 Hz), but did not affect those to isoprenaline. $Uptake_2$ inhibition by metanephrine (40 $\mu g\ kg^{-1}\ min^{-1}$) enhanced cardiac responses to isoprenaline (0·05 to 1·0 $\mu g\ kg^{-1}$ i.v.), but did not significantly alter those to noradrenaline or nerve stimulation. Responses to all agonist interventions were increased by the combined administration of cocaine and metanephrine. Cocaine preferentially enhanced the positive chronotropic cardiac response to noradrenaline, but metanephrine did not differentiate between heart rate and contractility. These results have been discussed in the light of the mechanism of drug action involved.

The importance of noradrenaline uptake into the sympathetic nerves ($Uptake_1$) as the main process terminating the action of noradrenaline, is well recognized (see Iversen 1967, 1971, 1974). The role of extraneuronal uptake ($Uptake_2$) has not been investigated to the same extent, although it is known to play a significant part in terminating the responses to sympathomimetics in a variety of isolated tissues, including cardiac (Lightman & Iversen 1969; Picken & Jarrott 1975; Bell & Grabsch 1976) and vascular (Kalsner 1969a, 1969b; Kalsner et al 1975; Guimaraes & Paiva 1977) preparations. We are unaware, however, of any studies which assess the relative contributions of neuronal and extraneuronal uptake in the heart and cardiovascular system of the whole animal and have therefore investigated the effects of differential and combined blockade of $Uptake_1$ and $Uptake_2$ on cardiovascular responses of anaesthetized dogs to noradrenaline, isoprenaline and sympathetic nerve stimulation. Cocaine was used to inhibit $Uptake_1$ (Trendelenburg 1959; Hertting et al 1961; Burgen & Iversen 1965) and metanephrine to inhibit $Uptake_2$ (Iversen 1965, 1967).

A preliminary account of this work was given as a communication to the British Pharmacological Society (Brown et al 1977).

METHODS

Beagle dogs of either sex, 9·9–14·0 kg, were anaesthetized with sodium pentobarbitone (Sagatal, 30 mg kg^{-1}) given via a fore-leg vein. Anaesthesia was maintained by intermittent intravenous infusion of sodium pentobarbitone at 1·2 mg min^{-1}. Artificial respiration was maintained by a Palmer pump (19 strokes per min) via a cuffed endotracheal tube. A catheter-tip PO_2 transducer, (G. D. Searle and Co.; Soutter et al 1975) was introduced into the abdominal aorta via the left femoral artery for the continuous monitoring of arterial PO_2 and intermittent sampling for the measurement of blood PCO_2 and pH. Inspired air was enriched with oxygen as necessary to maintain resting arterial PO_2 in excess of 90 mm Hg, and adjusted in stroke volume to maintain other parameters within normal physiological limits (pH 7·36–7·44, PCO_2 34–44 mm Hg).

The chest was opened in the mid-line and the left ansa subclavia nerve isolated and placed on shielded bipolar platinum electrodes and immersed in liquid paraffin. When required, rectangular pulses of supramaximal voltage (usually about 15 V) and 5 ms duration were supplied by a Grass S88 stimulator at different frequencies for 45 s. Both left and right vago-sympathetic nerve trunks were sectioned in the neck.

Left intraventricular pressure was measured by means of a catheter tip pressure transducer (type PC 350, Millar Instruments Inc.) passed into the left ventricle via the left carotid artery and central aortic blood pressure measured by means of a second similar transducer positioned in the ascending aorta via the right femoral artery. Correct positioning of the transducers was confirmed by continuous

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monitoring of the pressure waveforms on an Airmec 4-channel oscilloscope. Amplification of the signals from the pressure transducers was subject to a 50 Hz filter. Parameters derived from the left ventricular pressure signal included the first differential, dp/dt, and an additional index of contractility, (dp/dt)/P (or dlnP/dt) where P is the instantaneous left ventricular pressure (Nejad et al 1969, 1971). dp/dt was obtained using a Devices differentiator (type 3640) with a response flat to 100 Hz and (dp/dt)/P was obtained using a log differentiator similar to that described by Grossman et al (1971). The maximum values of these parameters were used as indices of cardiac "contractility". A heart rate meter was triggered by the aortic blood pressure signal which was also meaned by electronic integration (Devices conditioning unit No. 3500) and the ECG (lead II) was routinely monitored throughout each experiment. All parameters were recorded on a Devices M19 8-channel pen recorder.

Drugs were dissolved in 0·9% NaCl immediately before use and solutions of catecholamines contained, in addition, 0·002% w/v ascorbic acid as an antioxidant and were stored in a refrigerator between doses. Drug administration was via cannulated hind- and fore-leg veins.

Experimental Design

After preparation, each animal was allowed approximately 60 min to achieve stable resting conditions. Preliminary experiments were performed on 3 dogs to determine the doses and infusion rates of uptake antagonists that would produce the desired effect on uptake without themselves causing marked cardiovascular effects. Following this, randomized dose- and frequency-response curves to isoprenaline, noradrenaline and nerve stimulation were established under control conditions and then after or during the following interventions: (i) Cocaine HCl, 5 mg kg^{-1} i.v. and subsequent doses of 1 mg kg^{-1} i.v. every 45 min. (ii) Metanephrine, 40 μg kg^{-1} min^{-1} i.v. (iii) Those animals having received cocaine were then treated with metanephrine as in (ii) during continued dosing with cocaine. (iv) Those animals having received metanephrine only were then treated with cocaine as in (i) during maintained infusion of metanephrine.

Thus, each animal served as its own control and was then subjected to both differential uptake blockade (cocaine or metanephrine only) and combined uptake blockade (cocaine + metanephrine, or metanephrine + cocaine).

Drugs and materials

(±)-Isoprenaline hydrochloride (Suscardia, Pharmax Ltd.); (−)-noradrenaline acid tartrate (Levophed, Sterling Winthrop Ltd.); (±)-metanephrine hydrochloride (Sigma Ltd.); cocaine hydrochloride (May & Baker Ltd); sodium pentobarbitone (Sagatal, May & Baker Ltd); sodium chloride, ascorbic acid (BDH Ltd.). Unless otherwise stated, quantities and doses are referred to in the text as amounts of "base". Statistical testing of results was by analysis of variance.

RESULTS

Resting levels of the cardiovascular parameters under consideration during the initial control periods and after each of the interventions were as listed in Table 1.

Table 1. Resting haemodynamic parameters before agonist interventions (n = 5).

	Blood pressure (mm Hg)			Heart rate	Left ventricular (mm Hg)	$\frac{(dp/dt)}{P}$
	Syst.	Dias.	Mean	(beats min^{-1})	(s^{-1})	(s^{-1})
Group I experiments						
Control	135	103·6	120	148·6	2400	15·9
s.e.m.	±5·9	±5·0	±5·7	±10·8	±407	±2·3
After metanephrine 40 μg kg^{-1} min^{-1}	157·6	119·2	138	133	1780	13·3
s.e.m.	±6·5	±4·1	±6·4	±12·1	±285	±1·4
After metanephrine and cocaine	155	121	138·6	132	1240	12·6
s.e.m.	±3·2	±3·4	±2·7	±7·5	±108	±1·8
Group II experiments						
Control	133	101·6	121	124·6	1880	19·5
s.e.m.	±6·6	±7·6	±7·0	±10·3	±256	±1·4
After cocaine 5 mg kg^{-1} and 1 mg kg^{-1} every 45 min i.v.	132	108	121	116·2	1140	15·2
s.e.m.	±7·7	±7·3	±6·0	±8·0	±92·1	±1·8
After cocaine + metanephrine	151	120	133	124·6	1540	17·6
s.e.m.	±5·0	±4·1	±5·9	±13·9	±199	±1·9

Effects of cocaine

In preliminary experiments it was found that an initial dose of cocaine HCl (5 mg kg^{-1} i.v.) followed by a further dose of 1 mg kg^{-1} i.v. every 45 min was required to block responses to tyramine and to potentiate noradrenaline. The effects of cocaine on dose-response curves to isoprenaline (0·05–1 μg kg^{-1}) and noradrenaline (0·1–2 μg kg^{-1}) and frequency response curves to cardiac nerve stimulation (1–20 Hz) on dp/dt max and heart rate are shown in Fig. 1. The effects of cocaine on blood pressure responses to noradrenaline and isoprenaline are shown in Fig. 2. Responses to isoprenaline were unaffected by cocaine on any parameter and at any dose level tested whereas all responses to noradrenaline were enhanced. The

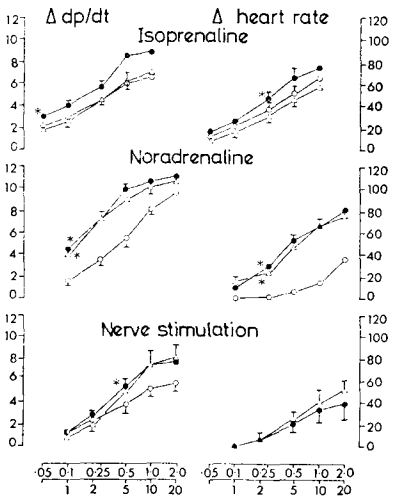

FIG. 1. Uptake blockade in anaesthetized dogs. Cardiac responses to isoprenaline, noradrenaline and sympathetic nerve stimulation under control conditions (○) and in the presence of cocaine, 5 mg kg^{-1} i.v. followed by 1 mg kg^{-1} every 45 min i.v. (△) and in the presence of cocaine, same dose, and metanephrine 40 $\mu g\ kg^{-1}\ min^{-1}$ i.v. (●). Each point is mean ± s.e.m. of 5 experiments; where no s.e.m. is shown this was within the size of the symbol. $*P < 0.05$ at this and all higher doses or frequencies of stimulation. Left ordinate: mm Hg $s^{-1} \times 10^3$. Right ordinate: heart rate (beats min^{-1}). Upper abscissa: $\mu g\ kg^{-1}$. Lower abscissa: Hz.

effects of nerve stimulation were increased by cocaine at frequencies of 5–20 Hz, but not at lower frequencies (Fig. 1). In 8 of the 10 control experiments no increase or a small fall in heart rate was observed at lower frequencies of stimulation of the left ansa subclavia nerve. This we have attributed to electrical spread to the vagus. However, after cocaine, only increases in heart rate in response to nerve stimulation were observed in all experiments.

Effects of metanephrine

Metanephrine was infused intravenously at rates of 10–100 $\mu g\ kg^{-1}\ min^{-1}$; no marked effects were observed on the cardiovascular system at less than 40 $\mu g\ kg^{-1}\ min^{-1}$ in any of the three dogs. Infusion at 40 $\mu g\ kg^{-1}\ min^{-1}$ produced small reductions in dp/dt and (dp/dt)/P, and raised the blood pressure (Table 1) and at 100 $\mu g\ kg^{-1}\ min^{-1}$ large increases in blood pressure coupled with falls in contractility indices were observed. At 10 and 20 $\mu g\ kg^{-1}\ min^{-1}$, metanephrine had no significant effects on the cardiovascular responses to isoprenaline or noradrenaline. At 40 and 100 $\mu g\ kg^{-1}\ min^{-1}$ it potentiated both isoprenaline and noradrenaline. The infusion rate of 40 $\mu g\ kg^{-1}\ min^{-1}$ thus combined a distinct enhancement of the cardiac response to catecholamines with minimal direct effects and was used as the standard rate in ensuing studies.

Metanephrine potentiated the positive inotropic responses to all doses of isoprenaline and the positive chronotropic responses to doses of 0·1 $\mu g\ kg^{-1}$ and above (Fig. 3). Positive inotropic responses to noradrenaline in doses of 0·25–2 $\mu g\ kg^{-1}$ and chronotropic responses to doses of 1–2 $\mu g\ kg^{-1}$ were also enhanced, but metanephrine alone did not affect cardiac responses to nerve stimulation.

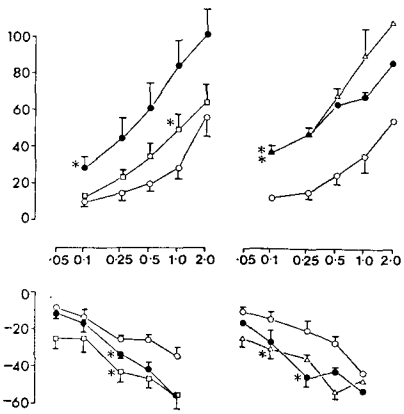

FIG. 2. Uptake blockade in anaesthetized dogs. Effects of noradrenaline (top) and isoprenaline (bottom) on blood pressure under control conditions (○) and in the presence of metanephrine (□), cocaine (△) and cocaine and metanephrine combined (●). Doses of cocaine and metanephrine as in Fig. 1. In the left hand column are the results of those experiments in which metanephrine alone was infused before treatment with both uptake antagonists; in the right hand column cocaine alone was administered first. Each point is mean ± s.e.m. of 5 experiments; where no s.e.m. is shown this was within the size of the symbol. $*P < 0.05$ at this and all higher doses. Ordinate: Δ b.p. (mm Hg). Abscissa: $\mu g\ kg^{-1}$.

Effects of combined cocaine and metanephrine

Cardiovascular responses to noradrenaline or nerve stimulation obtained after giving cocaine were not further modified by the added administration of metanephrine (Fig. 1). Likewise, responses to isoprenaline during an infusion of metanephrine were not further enhanced by the concurrent administration of cocaine (Fig. 3). However, subsequent administration of cocaine to those preparations already receiving metanephrine produced a further increase in cardiac responses to noradrenaline or nerve stimulation (Fig. 4) and subsequent infusion of metanephrine in those dogs which had already received cocaine, potentiated isoprenaline (Fig. 1).

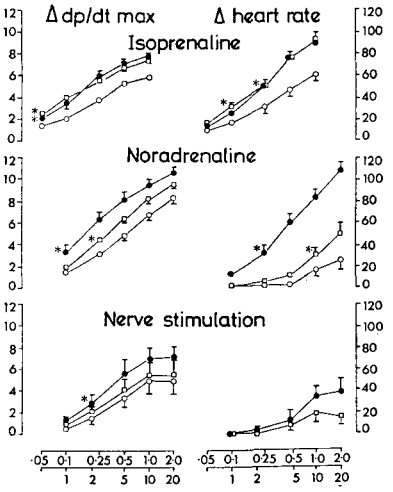

FIG. 3. Uptake blockade in anaesthetized dogs. Cardiac responses to isoprenaline, noradrenaline and sympathetic nerve stimulation under control conditions (○) and in the presence of metanephrine, 40 μg kg^{-1} min^{-1} (□) and in the combined presence of metanephrine, 40 μg kg^{-1} min^{-1} and cocaine, 5 mg kg^{-1} i.v. followed by 1 mg kg^{-1} i.v. every 45 min (●). Each point is mean ± s.e.m. of 5 experiments; where no s.e.m. is shown this was within the size of the symbol. * $P < 0{\cdot}05$ at this and all higher doses or frequencies of stimulation. Axes as in FIG. 1.

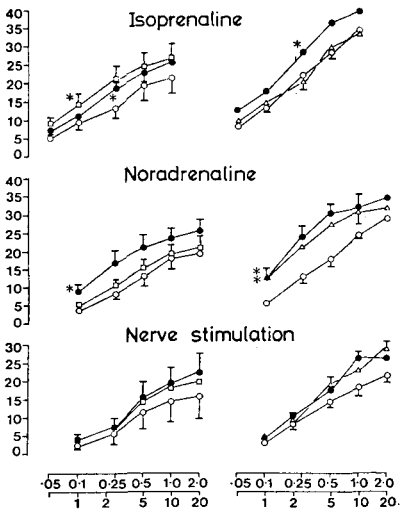

FIG. 4. Uptake blockade in anaesthetized dogs. Effects of isoprenaline, noradrenaline and sympathetic nerve stimulation on dp/dt/P under control conditions (○) and in the presence of metanephrine (□), cocaine (△) and combined metanephrine and cocaine (●). Doses of cocaine and metanephrine as in Fig. 1. In the left hand column are the results of those experiments in which metanephrine was infused before treatment with both uptake antagonists; in the right hand column cocaine alone was administered first. Each point is mean ± s.e.m. of 5 experiments; where no s.e.m. is shown this was within the size of the symbol. * $P < 0{\cdot}05$ at this and all higher doses and frequencies of stimulation. Ordinate: Δ(dp/dt)/P (s^{-1}). Upper abscissa: μg kg^{-1}. Lower abscissa: Hz.

Effects of cocaine and metanephrine on blood pressure responses and consequent effects on contractility indices

The effects of the various interventions made on pressor responses to noradrenaline and depressor responses to isoprenaline are shown in Fig. 2. Cocaine enhanced responses to noradrenaline throughout the dose-range used ($P < 0{\cdot}05$), but these responses were not further affected by the combined administration of cocaine and metanephrine. The pressor response to noradrenaline was enhanced by metanephrine, only at a dose of 1 μg kg^{-1}, but all responses were subsequently potentiated by concurrent administration of cocaine. Falls in blood pressure in response to isoprenaline were potentiated by individual treatment with either cocaine or metanephrine and the combined administration of uptake antagonists did not materially alter these modified responses.

Changes in cardiac afterload resulting from the effects of the interventions used on peripheral vascular resistance may influence dp/dt max in the absence of any inotropic effects. A further index of inotropic activity (dp/dt)/P, was therefore used which compensates for changes in cardiac afterload and is also less susceptible to changes in cardiac preload. When this measurement was utilized, metanephrine, whether administered alone, or in conjunction with cocaine, clearly enhanced the positive inotropic responses to isoprenaline (Fig. 4). The effects of the various interventions on responses to nerve stimulation in terms of (dp/dt)/P were qualitatively similar to those described previously for dp/dt max.

DISCUSSION

Inhibition of Uptake$_1$ by cocaine potentiated all of the cardiovascular responses to noradrenaline and to sympathetic nerve stimulation but, in the dose used, enhanced only the depressor response to isoprenaline. The criteria used for inhibition of Uptake$_1$ by cocaine were blockade of the pressor response to the indirectly acting sympathomimetic amine tyramine and potentiation of the responses to noradrenaline. Plots of changes in dp/dt max against changes in heart rate for noradrenaline show a striking shift in the relationship to higher heart rates when noradrenaline was administered in the presence of cocaine (see Fig. 5). No such shift occurred with isoprenaline and

cocaine. Similar observations in dog right-heart bypass preparations were reported by Furnival et al (1971). It was also established that metanephrine clearly enhanced all cardiovascular responses to isoprenaline. Superficial examination of the dose response curves suggested a slight preferential effect on dp/dt max since a statistically significant increase in this response occurred at a lower dose of isoprenaline (0·05 μg kg^{-1}) than a comparable potentiation of the heart rate response (0·1 μg kg^{-1}). Nevertheless, plots of dp/dt max against heart rate for isoprenaline (Fig. 5) demonstrate no real change in their relationship under any conditions of uptake blockade. Metanephrine was also found to enhance the cardiac responses to noradrenaline. The effects of noradrenaline and isoprenaline on dp/dt max may have been influenced by the changes in blood pressure produced by them in addition to their direct effect on cardiac contractility. To reduce this source of error, the measure (dp/dt)/P was also used. This function, where P is the instantaneous left ventricular pressure, has been proposed as an inotropic index less susceptible to pre- and afterload modification and potentially more indicative of changes in cardiac contractility as defined by V_{max}, i.e. the maximum velocity of shortening of the contractile elements at zero load (e.g. Nejad et al 1971).

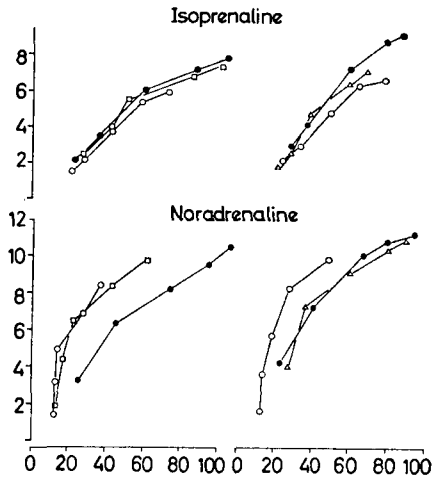

FIG. 5. Uptake blockade in anaesthetized dogs. The relationships between dp/dt max and heart rate in response to isoprenaline (0·05–1 μg kg^{-1}) and noradrenaline (0·1–2 μg kg^{-1}) under control conditions (○) and in the presence of metanephrine (□), cocaine (△) and combined metanephrine and cocaine (●). Doses of metanephrine and cocaine as in Fig. 1. In the left-hand column are the results of those experiments in which metanephrine was infused before treatment with both uptake antagonists; in the right-hand column cocaine alone was administered first. Ordinate: △ dp/dt mm Hg s^{-1} × 10^3. Abscissa: △ heart rate (beats min^{-1}).

When this index was used, the potentiation of the "inotropic" effect of noradrenaline by metanephrine was no longer seen. It is possible, therefore, that the enhanced effect on dp/dt max was secondary to a potentiation of the pressor response to noradrenaline by metanephrine. This possibility is supported by the lack of effect of metanephrine on the inotropic response to stimulation of the left ansa subclavia nerve. In this instance, none of the inotropic indices is affected, though positive chronotropic effects are apparently enhanced. However, this effect might also be artifactual, since under the conditions used in this series of experiments, several of the animals responded to nerve stimulation with a fall in heart rate. This we have attributed to spread of electrical excitation to the vagus nerve, and thus metanephrine could be acting by blocking the effects of vagal stimulation on the heart. We have not tested this possibility.

With the exception of the anomalies outlined above, it would seem that blockade of $Uptake_1$ with cocaine preferentially enhances the chronotropic response to noradrenaline, but that blockade of $Uptake_2$ with metanephrine potentiates both inotropic and chronotropic responses to isoprenaline to a similar degree. It is hard to assign any biological significance to the very slight shift toward the inotropic properties of isoprenaline in the presence of metanephrine. Our observations have been based on the assumption that the effects of cocaine and metanephrine are due predominantly to inhibition of neuronal and extraneuronal uptake processes respectively. If this is so, then our results lead to the conclusion that with isoprenaline, extraneuronal uptake is equally significant in the SA nodal tissue and in the ventricular myocardium. Confirmation is provided, however, for the considerably greater significance of neuronal uptake in the termination of positive chronotropic responses to exogenous and endogenous noradrenaline.

Blockade of either uptake process would be expected to increase the concentrations of the appropriate agonist drug available for receptor interaction. This, in turn, should lead to a parallel shift of the relevant dose-response curves to the left. The shifts observed in the present experiments are not parallel, but divergent and to the left. According to the criteria described by Kalsner (1974) this would suggest an alternative to uptake blockade as a mechanism of potentiation, since the potentiation seen is possibly dependent on the level of response

and not the local concentration of agonist. Unfortunately, the preparation used on this occasion is not suitable for the establishment of maximal responses to sympathomimetics and therefore we could not determine the effects of metanephrine or cocaine on these. It therefore remains a possibility that some of the potentiation observed to either of these agents is by an alternative mechanism.

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