Br. J. Pharmac. (1979), **67**, 283–292

EFFECTS OF CLONIDINE, PRAZOSIN AND PHENTOLAMINE ON HEART RATE AND CORONARY SINUS CATECHOLAMINE CONCENTRATION DURING CARDIOACCELERATOR NERVE STIMULATION IN SPINAL DOGS

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1 In spinal dogs, continuous electrical stimulation of the cardioaccelerator nerve produced a transient rise in aortic blood pressure and a sustained increase in both heart rate and coronary sinus blood flow. The latter effects were accompanied by a significant elevation in the coronary sinus plasma noradrenaline concentration without significant changes in the levels of dopamine and adrenaline. The concentrations of the three catecholamines in thoracic aorta plasma were not significantly changed by cardioaccelerator nerve stimulation.

2 Clonidine (20 μg/kg, i.v.), given during cardioaccelerator nerve stimulation, increased both mean aortic blood pressure and coronary sinus blood flow and decreased heart rate and coronary sinus venous plasma noradrenaline overflow.

3 Phentolamine (0.3 mg/kg, i.v.) completely antagonized these effects of clonidine. Prazosin (0.3 mg/kg, i.v.) inhibited by only 43 and 38% the respective reductions in heart rate and noradrenaline overflow elicited by clonidine.

4 On termination of cardioaccelerator stimulation (about 10 min after either prazosin or phentolamine), heart rate and coronary sinus noradrenaline overflow returned to control prestimulation levels.

5 Phentolamine or prazosin, administered alone during stimulation of the cardioaccelerator nerve, increased heart rate and noradrenaline overflow into the coronary sinus plasma. However, intravenous phentolamine and prazosin, in contrast to desipramine (0.3 mg/kg, i.v.) or tyramine (1.0 mg, i.a.), failed to change the tachycardia resulting from the local administration of noradrenaline into the sinus node artery (i.a.).

6 These results show that in spinal dogs the clonidine-induced reduction in heart rate (elevated by electrical stimulation of the cardioaccelerator nerve) is accompanied by a fall in the quantity of noradrenaline overflowing into the coronary sinus plasma. The latter effect is presumably the result of an action of clonidine on cardiac presynaptic α-adrenoceptors, the activation of which is followed by a reduction in the release of noradrenaline per nerve impulse. Phentolamine and prazosin are both antagonists of cardiac presynaptic α-adrenoceptors in spinal dogs, as suggested by their action against clonidine and by their positive chronotropic effect when administered during stimulation of the cardioaccelerator nerve.

Introduction

Much evidence favours the view that α-adrenoceptors are present on sympathetic nerve terminals and modulate neurotransmitter release via a classical feed-back mechanism, operating in response to the noradrenaline concentration within the synapse. It is postulated that activation of these presynaptic α-adrenoceptors results in a diminution of noradrenaline liberated per nerve impulse and this effect in turn is accompanied by a smaller end organ response. The inverse occurs when the modulatory function of presynaptic α-adrenoceptors is impaired with specific antagonists (see reviews: Langer, 1977; Starke, 1977; Westfall, 1977). This feed-back mechanism has been

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0007-1188/79/100283-10$01.00

extensively studied by measurement of the release of tritiated noradrenaline in numerous isolated perfused organs (see above reviews). However, due to the difficulty of both maintaining intact animal preparations for a prolonged period and measuring endogenous noradrenaline overflow, *in vivo* techniques have been sparingly used to investigate presynaptic mechanisms. Recently, however, Yamaguchi, De Champlain & Nadeau (1977) found that in anaesthetized dogs, heart rate increases elicited by short term electrical stimulation of cardiac sympathetic fibres were accompanied by a rise in the total catecholamine concentration overflowing into coronary sinus blood. Clonidine inhibited both these effects.

In the present study we have used the spinalized dog preparation for two reasons. Firstly, spinalization removes the major homeostatic mechanisms regulating the cardiovascular system, thus avoiding elevated levels of circulating catecholamines as observed in the preparation used by Yamaguchi *et al.* (1977). Secondly, we have shown that in the spinalized preparation continuous electrical stimulation of the cardioaccelerator nerve produces a stable, sustained positive chronotropic effect providing excellent steady-state conditions in which to investigate cardiac presynaptic α-adrenoceptors (Roach, Lefèvre & Cavero, 1978a).

The purpose of this study was to assess the effects of clonidine, a known potent agonist of presynaptic α-adrenoceptors, on the amounts of adrenaline, dopamine and noradrenaline overflowing into coronary sinus blood during sustained electrical stimulation of postganglionic cardioaccelerator fibres. In addition, the effects of phentolamine, a classical α-adrenoceptor antagonist (blocking both pre- and postsynaptic α-adrenoceptors; see above reviews), and prazosin, a compound reported to possess cardiac presynaptic α-adrenoceptor blocking properties in the dog (Roach *et al.*, 1978a; Constantine, Weeks & McShane, 1978), but not in the rat, as assessed against clonidine (Cavero, Lefèvre & Roach, 1977; Roach *et al.*, 1978a; Hua & Moulds, 1978), were examined on the tachycardia and noradrenaline overflow which result from cardiac sympathetic nerve stimulation.

Methods

Mongrel dogs of either sex weighing 15 to 18 kg were anaesthetized with sodium pentobarbitone (35 mg/kg, i.v.) and after tracheal intubation were ventilated with room air by means of a Bird Mark 7 respirator.

Catheters were placed in the thoracic aorta, via a brachial artery, and a brachial vein in order respectively to measure aortic blood pressure with a Statham P23Db pressure transducer and administer drugs intravenously. Heart rate was calculated with a cardiotachometer (custom made with 1% maximal error)

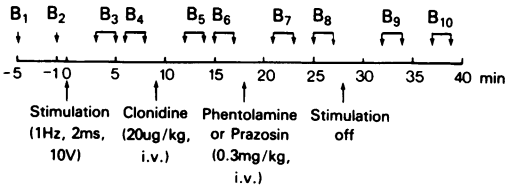

Figure 1 Flow-chart of the experimental procedure. B_1-B_{10} indicate the times at which thoracic aortic and coronary sinus blood samples were taken.

triggered from the electrocardiogram (lead II). The aortic blood pressure (Hellige MA preamplifier), heart rate and coronary sinus blood flow (Carolina Medical Electronics) were recorded on an electrostatic writer (Varian, Statos IV).

The spinal cord was exposed between the second and third cervical vertebrae and transected 5 min after local administration of lignocaine (2% w/v). The animals were then bivagotomized and the common carotid arteries ligated.

After thoracotomy (second intercostal space) the right stellate ganglion was fully decentralized and its most caudal post-ganglionic branch was placed on a bipolar platinum electrode. Then, a suitable period was allowed for blood pressure and heart rate stabilization before the administration of heparin (750 u/kg, i.v.). The coronary sinus was catheterized with a rubber cannula (french size 16 with closed round tip and 2 large eyes on the lateral wall by the tip) via the jugular vein. Coronary sinus blood passed through an extracorporeal (non cannulating) electromagnetic flow probe (the accuracy of which was verified by collecting blood samples over a minute period into a graduated cylinder) and drained into a container placed in a water bath maintained at 40°C. Coronary sinus blood samples could, therefore, be easily taken when required. Dextran (6% w/v) was added to replace the blood samples taken as described below. A roller pump (Sarns) was used to return the blood to the animal via a femoral vein.

In a group of spinal dogs the sinus node artery was dissected (Hashimoto, Tanaka, Hirata & Chiba, 1967) and a shunt was made between it and the carotid artery. The shunting circuit contained an extracorporeal electromagnetic flow probe and a rubber connector to allow drug administration into the sinus node.

Measurement of plasma catecholamines

Blood samples (2 to 3 ml) were collected from the thoracic aorta and the coronary sinus and then placed in ice-cooled plastic tubes. The blood was centrifuged at 500 *g* for about 15 min in a refrigerated centrifuge. The plasma was transferred into vials and frozen at −70°C until the catecholamines (noradrenaline,

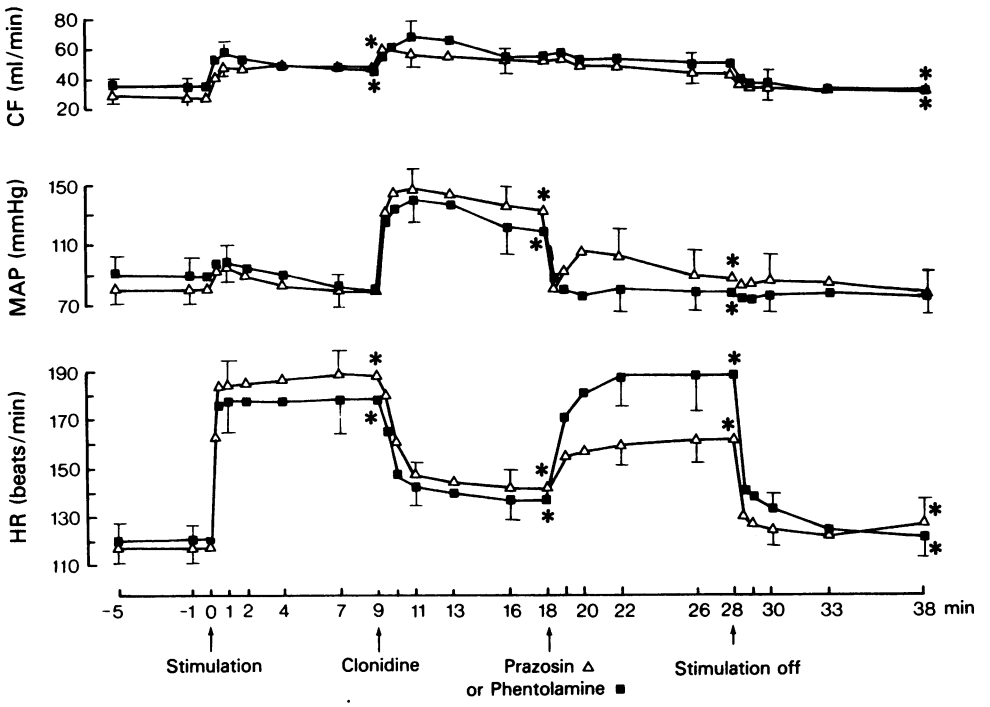

Figure 2 Effects of continuous stimulation of the cardioaccelerator nerve and successive treatment with clonidine (20 μg/kg, i.v.) and either phentolamine (■) or prazosin (△) (0.3 mg/kg, i.v.) on coronary sinus blood flow (CF), mean aortic blood pressure (MAP) and heart rate (HR) in two groups of spinal dogs (n = 5/group). Electrical stimulation was terminated 10 min after administration of the antagonists. *Indicates that the steady state response is significantly different from the immediately preceding treatment ($P < 0.05$, analysis of variance).

dopamine and adrenaline) were measured. Eight parts of plasma were mixed with one part 2 N perchloric acid solution containing 7% (in mol) $MgCl_2$ and 1% EGTA. After centrifugation (50,000 g) for 10 min in a Sorvall refrigerated centrifuge, 600 μl of the supernatant fluid was used for the determination of catecholamines by a radioenzymatic method (Da Prada & Zürcher, 1976), which allowed as little as 1 pg/assay sample of noradrenaline and adrenaline and 6 pg/assay sample of dopamine to be detected. All values were corrected for recovery by use of internal standards. The quantities of noradrenaline present in the coronary venous plasma are given as pg/ml.

Experimental procedure

The cardioaccelerator nerve was stimulated continuously (1.0 Hz, 2.0 ms, 10V) with a F. Haer Digital Stimulator (model Pulsar 6i). When the heart rate increase reached a steady state, clonidine (20 μg/kg) was administered intravenously followed a few minutes later by either phentolamine (0.3 mg/kg, i.v., n = 5) or prazosin (0.3 mg/kg, i.v., n = 5). When the heart rate response to the latter two compounds became stable the electrical stimulation was stopped. Figure 1 shows the experimental procedure and indicates the times when blood samples were taken.

The effects of prazosin or phentolamine on heart rate and coronary sinus catecholamine concentration were also studied during electrical stimulation of the cardioaccelerator nerve in dogs, to some of which clonidine was subsequently administered in a dose of 20 μg/kg. Finally, the effects of phentolamine, prazosin or desipramine (0.3 mg/kg, i.v., n = 3) were studied on the sustained heart rate increase (△ 50 to 60 beats/min) produced by an infusion of noradrenaline (3.0 to 10.0 ng kg^{-1} min^{-1}) into the sinus node of spinal dogs. The effect on heart rate of tyramine (1.0 μg, total dose; n = 2) injected into the sinus node artery was also examined.

Statistical analysis

The data are given as means ± standard errors of the means. The significance of responses to various treatments was tested by use of a two-way analysis of variance.

Table 1 Noradrenaline (NA) dopamine (DA) and adrenaline (Ad) concentrations (pg/ml) measured in plasma from coronary sinus and thoracic aorta in two groups of spinal dogs ($n = 5$)

Plasma catecholamines		n	*Baseline 1*	*Stim*	*Clonidine*	*Clonidine + prazosin*	*Baseline 2*	n	*Baseline 1*	*Stim*	*Clonidine*	*Clonidine + phentol*	*Baseline 2*
Coronary	NA	5	60 ± 28	329 ± 43*	111 ± 23*†	236 ± 41*§	52 ± 21	5	155 ± 39	383 ± 78*	141 ± 25†	498 ± 57*§	137 ± 26
	DA	5	17 ± 7	21 ± 4	18 ± 4	27 ± 8	14 ± 4	5	50 ± 20	27 ± 4	28 ± 7	50 ± 14	37 ± 12
	Ad	5	30 ± 8	40 ± 5	43 ± 14	44 ± 9	25 ± 7	5	48 ± 13	52 ± 12	42 ± 13	158 ± 89	64 ± 21
Aortic	NA	5	25 ± 10	25 ± 9	20 ± 13	69 ± 40	35 ± 17	5	68 ± 32	99 ± 29	57 ± 24	118 ± 38	98 ± 33
	DA	5	23 ± 7	34 ± 12	29 ± 16	19 ± 5	47 ± 19	5	28 ± 9	33 ± 15	22 ± 7	39 ± 18	27 ± 8
	Ad	5	43 ± 20	34 ± 17	26 ± 14	23 ± 7	45 ± 20	5	30 ± 10	25 ± 7	38 ± 14	86 ± 55	92 ± 54

The flow-chart of the experimental procedure is illustrated in Figure 1. Each column shows the mean ± s.e. mean calculated by using the averages of two sequential plasma samples obtained within each of the 5 experimental procedures (see Figure 2). Analysis of variance indicated that there was no significant difference between the combined samples. Baseline 1 shows the values before starting stimulation (Stim) and values after clonidine (20 μg/kg, i.v.) alone or followed by either prazosin or phentolamine (0.3 mg/kg, i.v.) are also given. Baseline 2 values were obtained after terminating stimulation and were not different from Baseline 1. The analysis of variance on coronary sinus dopamine and adrenaline as well as on the catecholamines from aortic plasma show no significant difference between all the treatments.

* Significant difference ($P < 0.05$: analysis of variance) from Baseline 1.

† Significant difference ($P < 0.05$: analysis of variance) from Stim.

§ Significant difference ($P < 0.05$: analysis of variance) from Clonidine.

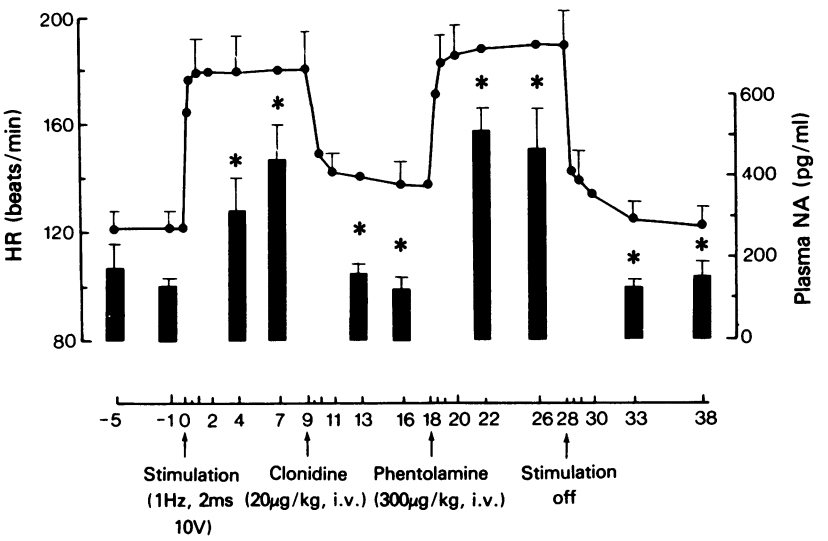

Figure 3 Heart rate (HR, ●) and coronary sinus plasma noradrenaline (NA) overflow (solid columns) in spinal dogs (n = 5) in which clonidine (20 μg/kg, i.v.) and phentolamine (0.3 mg/kg, i.v.) were studied during electrical stimulation of the cardioaccelerator nerve. *Indicates that the values of both parameters are significantly different from those of the immediately preceding treatment ($P < 0.05$, analysis of variance).

A correlation analysis was performed to test if there was a significant linear relationship between heart rate levels and coronary sinus plasma noradrenaline concentrations.

Drugs

The drugs used in this study were clonidine hydrochloride (Boerhinger Ingheleim), desipramine hydrochloride (Ciba-Geigy), dextran-clin solution (Clin-Comar-Bila Laboratories), lignocaine hydrochloride (Xylocaine 2%, Roger Bellon), noradrenaline bitartrate (Sigma), phentolamine mesylate (Ciba-Geigy), prazosin base (synthesized by our Chemistry Dept.: for the method used to dissolve prazosin, see Roach, Gomeni, Mitchard, Nicolas & Cavero, 1978), tyramine hydrochloride (Sigma) and pentobarbitone sodium (Abbott).

The doses of clonidine, desipramine, noradrenaline, phentolamine, prazosin and tyramine in the text refer to the bases of these compounds.

Results

Effects of electrical stimulation of cardioaccelerator nerve on several cardiovascular parameters and plasma catecholamines

Electrical stimulation of the cardioaccelerator nerve produced a rapidly developing tachycardia which attained a steady state within about 3 min (Figures 2, 3, 4 and 6) and remained at this level for the duration of the experimental procedure (Figure 6). Coronary blood flow also increased significantly (Figure 2). Mean aortic blood pressure increased during the first 2 min of stimulation and then declined to prestimulation levels (Figure 2).

The noradrenaline concentration in the coronary sinus plasma was greater than that in the thoracic aortic plasma under control conditions (Table 1). However, no venous/arterial gradient was observed for dopamine or adrenaline (Table 1). The basal coronary sinus plasma noradrenaline concentration (Table 1) was higher in the group of dogs used to study phentolamine than those given prazosin, but the changes produced by stimulation of cardiac accelerator nerve were similar (Table 1). The variability of the baseline plasma noradrenaline content is possibly in part due to the use of mongrel dogs.

The concentration of noradrenaline in the coronary sinus venous plasma was increased by continuous electrical stimulation of the cardioaccelerator nerve (Table 1; Figures 3 and 4). Dopamine and adrenaline overflow were not significantly changed by the stimulation (Table 1). In contrast, there were no corresponding changes in thoracic aortic plasma catecholamine concentrations during cardioaccelerator nerve stimulation.

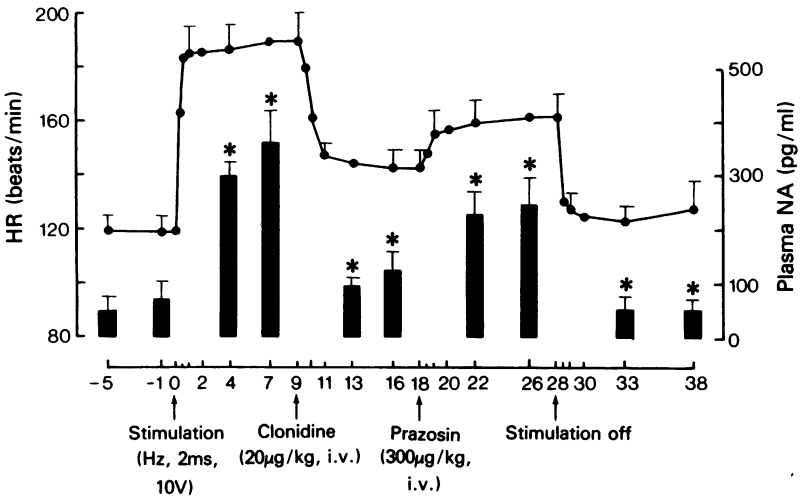

Figure 4 Heart rate (HR, ●) and coronary sinus plasma noradrenaline (NA) overflow (solid columns) in spinal dogs ($n = 5$) in which clonidine (20 μg/kg, i.v.) and prazosin (0.3 mg/kg, i.v.) were studied during electrical stimulation of the cardioaccelerator nerve. *Indicates that the values of both parameters are significantly different from those of the immediately preceding treatment ($P < 0.05$, analysis of variance).

Effects of clonidine followed by prazosin and phentolamine, on plasma catecholamines and on several cardiovascular parameters during cardioaccelerator nerve stimulation

Clonidine increased aortic blood pressure and coronary sinus blood flow (Figure 2) whereas it decreased the elevated heart rate (Figures 2, 3 and 4). The peak blood pressure effect of clonidine occurred earlier than the peak fall in heart rate (Figure 2). Clonidine reduced the coronary sinus plasma noradrenaline concentration from the sympathetically stimulated heart (Table 1; Figures 3 and 4). In the group of dogs used for the study of phentolamine, clonidine lowered coronary sinus plasma noradrenaline to levels similar to those measured before stimulation of the cardioaccelerator nerve (Table 1). In the group of animals used to study prazosin, clonidine reduced coronary sinus plasma noradrenaline. However, this value was significantly higher than that measured before nerve stimulation (Table 1). Coronary sinus plasma dopamine and adrenaline concentrations, as well as the catecholamine content of plasma sampled from the thoracic aorta, were not significantly changed by clonidine (Table 1).

At the time when phentolamine or prazosin were administered, the clonidine-induced pressor response was exponentially declining towards baseline levels. In contrast, the effect on heart rate was maximal and appeared to be in a steady state (Figure 2). Phentolamine reversed the clonidine-induced reduction of the sympathetic tachycardia (Figure 2) and this effect was accompanied by an increase in cardiac noradrenaline overflow to levels above those measured before clonidine administration (Figure 3). In addition, phentolamine accelerated the rate of decline of the pressor response to clonidine (Figure 2).

Administration of prazosin only partially reversed (43%) the fall in heart rate elicited by clonidine. This was accompanied by a similar partial increase (38%) in the noradrenaline concentration overflowing into the coronary sinus (Figure 4). Prazosin, like phentolamine, antagonized the clonidine pressor response (Figure 2).

Termination of the electrical stimulation of the cardioaccelerator nerve was followed by a complete restoration of the heart rate, coronary blood flow (Figure 2) and coronary sinus plasma noradrenaline content (Figures 3 and 4; Table 1) to prestimulation levels.

Effects of phentolamine and prazosin on coronary sinus plasma noradrenaline and heart rate during cardioaccelerator nerve stimulation

In dogs in which heart rate was increased by continuous electrical stimulation of the cardioaccelerator nerve, administration of phentolamine or prazosin (0.3 mg/kg, i.v.) further increased heart rate (Figures 5 and 6) and the concentration of noradrenaline measured in coronary sinus plasma (Figure 5). On suspension of stimulation 10 min after phentolamine or prazosin administration, heart rate and noradrena-

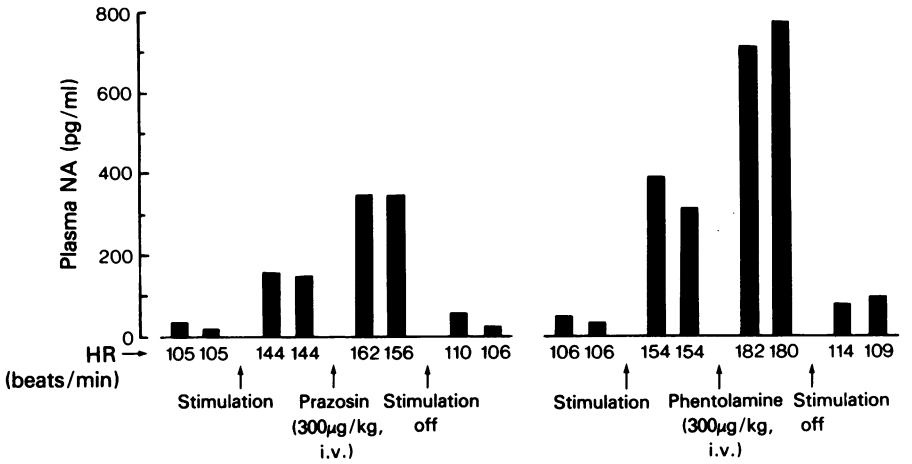

Figure 5 Effects of prazosin (n = 1) and phentolamine (n = 2) (both 0.3 mg/kg, i.v.) on heart rate and coronary sinus plasma noradrenaline (NA) overflow during electrical stimulation of the cardioaccelerator nerve in spinal dogs. The correlation between heart rate and coronary sinus plasma noradrenaline was highly significant for each animal as demonstrated in Figure 7 for similar experiments.

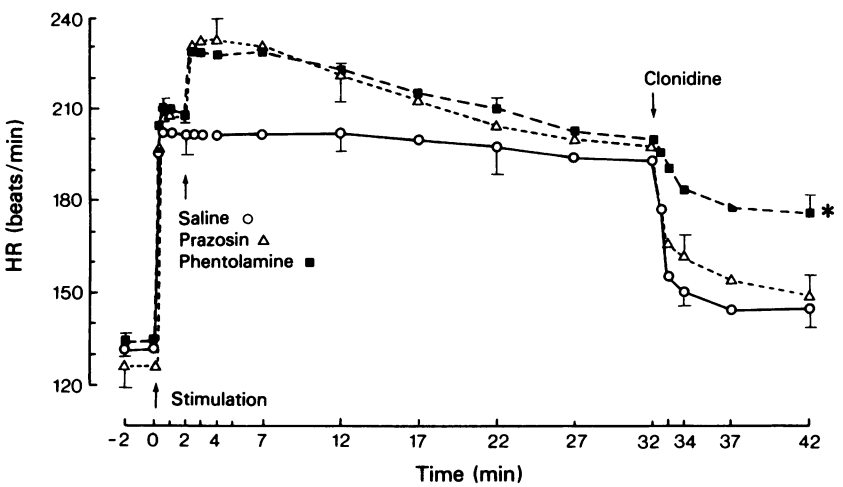

Figure 6 Effects of either phentolamine (■) or prazosin (△) (0.3 mg/kg, i.v.: n = 3/compound) or saline (○, n = 4) on heart rate increased by electrical stimulation of the cardioaccelerator nerve in spinal dogs. The effects of clonidine (20 μg/kg, i.v.) were studied 30 min after phentolamine, prazosin or saline. *Indicates that the response to clonidine in phentolamine-treated dogs is significantly smaller than in control animals ($P < 0.05$, analysis of variance).

line concentration returned to prestimulation levels (Figure 5).

Effects of clonidine on heart rate when administered 30 min after phentolamine and prazosin and during cardioaccelerator nerve stimulation

Phentolamine and prazosin given during continuous stimulation of the cardioaccelerator nerve further increased heart rate (Figure 6). The maximal effects of phentolamine and prazosin remained constant for 5 min after which they slowly declined towards pretreatment levels at rates of approximately 1.2 and 1.3 beats/min per min, respectively (Figure 6). In the matched control group there was no significant change in heart rate during the 30 min observation period. Administration of clonidine (20 μg/kg, i.v.) 30 min after phentolamine, produced a fall in heart rate which was both slower in onset, and smaller in magnitude, than the response observed in matched con-

trol animals given saline (Figure 6). In contrast, the clonidine-induced reductions in heart rate in the prazosin pretreated animals were not significantly different from those observed in the control group (Figure 6).

Effects of phentolamine and prazosin on noradrenaline-induced tachycardia

Noradrenaline (3.0 to 10.0 ng kg^{-1} min^{-1}) infused into the sinus node artery produced a stable (50 to 60 beats/min) increase in heart rate which persisted over 20 min. The intravenous administration of phentolamine or prazosin (0.3 mg/kg) failed to modify this elevated heart rate. However, an intravenous dose of desipramine further increased heart rate by 42 ± 9 beats/min (n = 3). Tyramine (1.0 μg, total dose) injected directly into the sinus node artery during the noradrenaline infusion also produced a further increase in heart rate (of 15 and 20 beats/min in two dogs).

Discussion

Electrical stimulation of postganglionic nerve fibres emerging from the right stellate ganglion induced an increase in the coronary sinus plasma noradrenaline concentration, although not in plasma obtained from the thoracic aorta. This confirms previous results (Yamaguchi, De Champlain & Nadeau, 1975; Yamaguchi *et al.*, 1977; Levy & Blattberg, 1977). This effect was not the direct consequence of increased myocardial activity since it has also been shown to occur under experimental conditions in which heart rate and cardiac output were kept constant (Siegel, Gilmore & Sarnoff, 1961).

As indicated by the highly significant correlation between heart rate and coronary venous plasma noradrenaline content (Figure 7), the increase in coronary sinus plasma noradrenaline concentration produced by electrical stimulation of the cardioaccelerator nerve may be considered as a biochemical index of cardiac sympathetic activity, generally measured as chronotropic and/or inotropic effects. It is evident that the increase in coronary sinus plasma noradrenaline did not exclusively, nor predominantly, originate from the sinus pacemaker region which, although richly innervated (Angelakos, 1965), receives only a minimal proportion of total coronary blood flow (MacLean, Hedenstrom & Kim, 1961). However, it is not unreasonable to assume that the changes in noradrenaline measured in the coronary venous plasma during electrical stimulation of the cardioaccelerator nerve reflect qualitatively, and possibly quantitatively, the changes in noradrenaline in the venous blood draining from the cardiac pacemaker region.

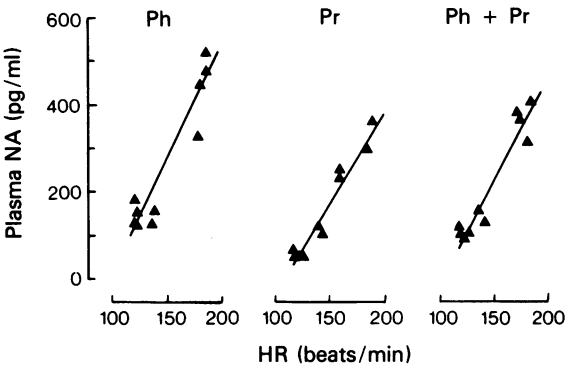

Figure 7 Heart rates plotted as function of coronary sinus plasma noradrenaline (NA) concentrations in two groups of dogs (n = 5) used to study phentolamine (Ph: see Figure 3) and prazosin (Pr: see Figure 4). The plot for the combined data of Ph and Pr is also given (Ph + Pr). There was a highly significant linear correlation (r = 0.9, d.f. 8; $P < 0.01$) between the two parameters. The slopes (Ph: 5.0 ± 0.3; Pr: 4.2 ± 0.4) of the straight lines fitting the data of the two separate groups were not significantly different (t test), indicating that the correlation was independent of the treatments.

Dopamine and adrenaline concentrations in both coronary sinus and aortic plasma did not change during cardioaccelerator stimulation confirming that the sympathetic neurotransmitter of the mammalian heart is noradrenaline. In addition, the absence of an elevation in aortic noradrenaline levels indicates that the electrical stimulation of the postganglionic sympathetic nerves in our experiments was localized to the myocardium.

Electrical stimulation of the cardioaccelerator nerve produced a tachycardia which persisted as long as stimulation was maintained. Under these experimental conditions, administration of clonidine produced a fall in heart rate. This effect was attributed to an inhibition of sympathetic stimulation at a neuronal level since: (1) the overflow of noradrenaline into coronary sinus plasma decreased and (2) clonidine did not modify the tachycardia produced by an intravenous infusion of noradrenaline, isoprenaline or tyramine (Cavero, Dennis, Roach & Scatton, 1979). The mechanism whereby clonidine affects noradrenaline release, would appear to involve stimulation of α-adrenoceptors on sympathetic nerve terminals. This results in a reduction in the amount of noradrenaline liberated per individual nerve impulse (see Introduction). In contrast, compounds blocking these receptors increase the release of neurotransmitter.

One aim of this study was to compare the effects of phentolamine and prazosin, two antagonists of postsynaptic vascular α-adrenoceptors (Lefèvre-Borg, Roach & Cavero, 1979a; Lefèvre-Borg, Gomeni,

Roach & Cavero, 1979b) on canine cardiac presynaptic α-adrenoceptors. There is evidence favouring a differential sensitivity of these modulatory receptors to a given agonist in different animal species at least *in vivo* (Roach *et al.*, 1978a; Roach, Lefèvre-Borg & Cavero, 1978b). Recently, it has been suggested that even presynaptic α-adrenoceptors of various organs within the same species may differ as far as their affinity for clonidine is concerned (Doxey & Everitt, 1977; Dubocovich, 1979).

In this study, phentolamine effectively and completely antagonized the reduction of the sympathetic tachycardia elicited by clonidine. This effect, which was accompanied by a return of the concomitantly reduced noradrenaline overflow to levels higher than those measured before clonidine administration, was neither due to an extramyocardial release of noradrenaline nor to an inhibition of neuronal noradrenaline uptake since no major change occurred in aortic plasma catecholamine levels. The latter mechanism was also excluded by the fact that phentolamine failed to increase further the heart rate which was already elevated by infusion of noradrenaline into the sinus node artery, although desipramine and tyramine did so. The results of this experiment suggest that the increased concentration of coronary sinus plasma noradrenaline, and the associated cardiac acceleration, were unlikely to be the result of a displacement of noradrenaline from α-adrenoceptors present in the cardiac pacemaker region.

Phentolamine enhanced heart rate and coronary sinus plasma noradrenaline when administered during stimulation of the cardioaccelerator nerve. Therefore, it may be possible that the observed antagonism of clonidine by phentolamine could be explained on this basis and not by the displacement of clonidine from receptor sites. However, phentolamine significantly reduced clonidine-induced negative chronotropic effects in animals in which its own heart rate effects had disappeared (see Figure 6).

As for phentolamine, the partial antagonism of clonidine by prazosin and the positive chronotropic effect of the latter compound given alone during cardioaccelerator nerve stimulation were due to blockade of cardiac presynaptic α-adrenoceptors and not to other mechanisms (as discussed above for phentolamine).

The fact that prazosin, in contrast to phentolamine, did not inhibit clonidine-induced bradycardia 30 min after its administration could be due to its rapid clearance from presynaptic α-adrenoceptors. An alternative explanation might be that the affinity of clonidine for these receptors is higher than that of prazosin, thus allowing an easier displacement of the latter compound from receptor sites.

In previous work we found that the dose of prazosin used in the present study was sufficient to inhibit completely the presynaptic effects of clonidine (Roach *et al.*, 1978a). However, in this investigation, prazosin was only partially effective. Thus, this finding concerning the activity of prazosin against clonidine is more in line with the report of Commarato, Langley, Dugan, Lattime, Smith, Tessman & Kaplan (1978), and differs quantitatively from our previous report (Roach *et al.*, 1978a) and that of Constantine *et al.* (1978). At present, we do not know the reason for this quantitative difference between two sets of experiments carried out at different periods. It may be possible that metabolites of prazosin possess stronger cardiac presynaptic α-adrenoceptor blocking activities than prazosin itself and the biotransformation rate of prazosin may change from animal to animal, especially, as in our case, when mongrel dogs are used.

In vitro experiments in which rabbit pulmonary arteries (Cambridge, Davey & Massingham, 1977) or guinea-pig atria (Commarato *et al.*, 1978) were used, indicate that prazosin possesses a very low affinity for both vascular and cardiac presynaptic α-adrenoceptors of these species. From these and other results (as discussed by Lefèvre-Borg *et al.*, 1979b) it has been suggested that presynaptic α-adrenoceptors differ from those located postsynaptically, for which prazosin has a pronounced affinity. The variability of the antagonist action of prazosin against the cardiac presynaptic α-adrenoceptors stimulation by clonidine could be explained if these receptors were made up of a mixed population of two types of α-adrenoceptor at present classified as 'presynaptic (α_2) and postsynaptic (α_1) α-adrenoceptors' (Berthelsen & Pettinger, 1977). The degree of blockade of the cardiac presynaptic stimulant action of clonidine induced by prazosin would then be proportional to the amount of receptors of the 'vascular postsynaptic type' (susceptible to the blocking action of prazosin) present on the cardiac sympathetic nerve terminal. The fact that phentolamine is consistently effective in antagonizing the presynaptic effect of clonidine could be due to its relatively high affinity for both types of receptors. This hypothesis, of course, needs verification. However, it may not be so unlikely if one considers that it is now accepted that varying proportions of both types of β-adrenoceptors (β_1- and β_2-) are present in the heart and bronchial tree (Åblad, Carlsson, Dahlof & Ek, 1976; Johnsson, 1976).

Prazosin appears to be less potent than phentolamine as an inhibitor of cardiac presynaptic α-adrenoceptors, as judged by their antagonism of the effect of clonidine in reducing the heart rate of dogs with a sympathetic tachycardia. However, phentolamine and prazosin were equally effective in enhancing heart rate when administered during stimulation of the cardioaccelerator nerve, suggesting that they possess similar cardiac α-adrenoceptor blocking activities in the absence of clonidine. Thus, the use of clonidine

or other agonists should not be envisaged as the only test to assess the cardiac presynaptic inhibitory properties of a compound.

The present results suggest that the measurement of noradrenaline concentration in the coronary sinus plasma of spinal dogs may be a useful *in vivo* approach for studying cardiac presynaptic mechanisms.

The authors thank Mrs J. Lorrain for her excellent technical assistance in some of the experiments. Thanks are also extended to Dr R. Gomeni for his help with the statistical analysis of the data and to Mrs N. Sieller for secretarial work.

References

ÅBLAD, B., CARLSSON, E., DAHLOF, C. & EK, L. (1976). Some aspects of the pharmacology of β-adrenoreceptor blockers. *Drugs*, **11**, Suppl. 1, 100–110.

ANGELAKOS, E.T. (1965). Regional distribution of catecholamines in the dog heart. *Circulation Res.*, **16**, 39–44.

BERTHELSEN, S. & PETTINGER, W.A. (1977). A functional basis for classification of α-adrenergic receptors. *Life Sci., Oxford*, **21**, 595–606.

CAMBRIDGE, D., DAVEY, M.J., MASSINGHAM, R. (1977). Prazosin, a selective antagonist of post-synaptic α-adrenoceptors. *Br. J. Pharmac.*, **59**, 514P.

CAVERO, I., DENNIS, T., ROACH, A.G. & SCATTON, B. (1979). Contribution of cardiac presynaptic α-adrenoceptors to the clonidine-induced bradycardia in dogs. In *Presynaptic Receptors.* ed. Langer, S.Z., Starke, K. & Dubocovich, M.L. pp. 53–57. Oxford: Pergamon Press.

CAVERO, I., LEFÈVRE, F. & ROACH, A.G. (1977). Differential effects of prazosin on the pre- and postsynaptic α-adrenoceptors in the rat and dog. *Br. J. Pharmac.*, **61**, 469P.

COMMARATO, M.A., LANGLEY, A.E., DUGAN, D.H., LATTIME, E.C., SMITH, R.D., TESSMAN, D.K. & KAPLAN, H.R. (1978). Prazosin and phentolamine: Comparative cardiovascular and autonomic profile. *Clin. exp. Hypertension*, **1**, 191–217.

CONSTANTINE, J.W., WEEKS, R.A. & MCSHANE, W.K. (1978). Prazosin and presynaptic α-receptors in the cardioaccelerator nerve of the dog. *Eur. J. Pharmac.*, **50**, 51–60.

DA PRADA, M. & ZÜRCHER, G. (1976). Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomole range. *Life Sci., Oxford*, **19**, 1161–1174.

DOXEY, J.C. & EVERITT, J. (1977). Inhibitory effects of clonidine on responses to sympathetic nerve stimulation in the pithed rat. *Br. J. Pharmac.*, **61**, 559–566.

DUBOCOVICH, M. (1979). Pharmacological differences between the alphapresynaptic adrenoceptors in the peripheral and the central nervous system. In *Presynaptic Receptors.* ed. Langer, S.Z., Starke, K. & Dubocovich, M.L. pp. 29–36. Oxford: Pergamon Press.

HASHIMOTO, K., TANAKA, S., HIRATA, M. & CHIBA, S. (1967). Responses of the sino-atrial node to change in pressure in the sinus node artery. *Circulation Res.*, **21**, 297–304.

HUA, A.S.P. & MOULDS, R.F.W. (1978). The effect of prazosin on pre- and postsynaptic α-receptors in the pithed rat. *Clin. exp. Pharmac. Physiol.*, **5**, 525–528.

JOHNSSON, G. (1976). Use of β-adrenoreceptor blockers in combination with β-stimulators in patients with obstructive lung diseases. *Drugs*, **11**, Suppl. 1, 171–176.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60**, 481–497.

LEFÈVRE-BORG, F., GOMENI, R., ROACH, A.G., CAVERO, I. (1979b). Mechanism of antihypertensive activity of orally administered prazosin in spontaneously hypertensive rats. *J. Cardiovasc. Pharmac.*, **1**, 31–42.

LEFÈVRE-BORG, F., ROACH, A.G. & CAVERO, I. (1979a). Comparison of cardiovascular actions of dihydralazine, phentolamine and prazosin in spontaneously hypertensive rats. *J. Cardiovasc. Pharmac.*, **1**, 19–29.

LEVY, M.N. & BLATTBERG, B. (1977). Correlation of the mechanical responses of the heart with the norepinephrine overflow during cardiac sympathetic neural stimulation in the dog. *Cardiovasc. Res.*, **11**, 481–488.

MACLEAN, L.D., HEDENSTROM, P.H. & KIM, Y.S. (1961). Distribution of blood flow to the canine heart. *Proc. Soc. exp. Biol. Med.*, **107**, 786–789.

ROACH, A.G., GOMENI, R., MITCHARD, M., NICOLAS, J.P. & CAVERO, I. (1978). The blood pressure lowering effects of intravenous versus intracerebroventricular prazosin in anaesthetised cats. *Eur. J. Pharmac.*, **49**, 271–278.

ROACH, A.G., LEFÈVRE, F. & CAVERO, I. (1978a). Effects of prazosin and phentolamine on cardiac presynaptic α-adrenoceptors in the cat, dog and rat. *Clin. exp. Hypertension*, **1**, 87–101.

ROACH, A.G., LEFÈVRE-BORG, F. & CAVERO, I. (1978b). Responsiveness of cardiac presynaptic α-adrenoceptors to clonidine in the cat, dog, guinea pig, rabbit and rat. In *Recent Advances in the Pharmacology of Adrenoceptors.* ed. Szabadi, E., Bradshaw, C.M. & Bevan, P. pp. 353–354. Amsterdam: Elsevier North-Holland Biomedical Press.

SIEGEL, J.H., GILMORE, J.P. & SARNOFF, S.J. (1961). Myocardial extraction and production of catecholamines. *Circulation Res.*, **9**, 1336–1350.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

WESTFALL, T.A. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.*, **57**, 659–728.

YAMAGUCHI, N., DE CHAMPLAIN, J. & NADEAU, R. (1975). Correlation between the response of the heart to sympathetic stimulation and the release of endogenous catecholamines into the coronary sinus of the dog. *Circulation Res.*, **36**, 662–668.

YAMAGUCHI, N., DE CHAMPLAIN, J. & NADEAU, R. (1977). Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. *Circulation Res.*, **41**, 108–117.

(Received January 30, 1979.
Revised March 1, 1979.)