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Evidence for a peripheral component in the sympatholytic effect of clonidine in rats

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1 In an attempt to assess separately the peripheral and central effects of clonidine on cardiovascular parameters and plasma catecholamine levels, the selective α_2-adrenoceptor antagonist idazoxan (RX 781094) was given either intravenously (i.v.) or intracerebroventricularly (i.c.v.) to anaesthetized rats before administration of intravenous clonidine. Plasma noradrenaline and plasma growth hormone concentrations were used as indices of peripheral sympathetic nervous activity and central α-adrenoceptor stimulation, respectively.

2 Peripheral and central administration of idazoxan antagonized the cardiovascular responses to i.v. clonidine, 5 $\mu g\,kg^{-1}$. However, idazoxan was more effective against the hypotension than the bradycardia induced by clonidine.

3 Idazoxan 300 $\mu g\,kg^{-1}$ i.v. and 50 μg i.c.v. prevented clonidine-induced falls in plasma noradrenaline and adrenaline. The results suggest that 50 μg idazoxan i.c.v. caused some blockade of peripheral as well as central α_2-adrenoceptors. Idazoxan, 10 μg i.c.v., caused similar inhibition of the hypotensive response to clonidine as 300 $\mu g\,kg^{-1}$ i.v. and 50 μg i.c.v. but did not significantly inhibit the clonidine-induced fall in plasma noradrenaline concentration.

4 Animals pretreated with i.v. or i.c.v. idazoxan had significantly lower levels of plasma growth hormone than vehicle-treated rats. Idazoxan 10 μg and 50 μg i.c.v. suppressed growth hormone secretion to the same extent.

5 These results suggest that stimulation of peripheral, prejunctional α_2-adrenoceptors in anaesthetized rats may contribute to the fall in plasma catecholamines produced by i.v. clonidine, and confirm that the hypotensive effect is centrally mediated.

Introduction

The hypotensive effect of clonidine is usually thought to be initiated by stimulation of α_2-adrenoceptors at pontomedullary sites within the central nervous system (Schmitt, 1977; Kobinger, 1978; Van Zwieten & Timmermans, 1979; Timmermans *et al.*, 1981). Clonidine can also reduce stimulation-evoked release of noradrenaline from various isolated tissues through an action at prejunctional α_2-adrenoceptors (see Starke, 1977). The aim of the present study was therefore to investigate whether stimulation of peripheral prejunctional α_2-adrenoceptors in intact animals may contribute to the fall in plasma catecholamines produced by intravenous clonidine.

Recent studies *in vitro* with isolated tissues of the rat (Chapleo *et al.*, 1981) and neuropharmacological studies in rats and mice (Dettmar *et al.*, 1983) have shown that idazoxan (RX781094) is a potent and highly selective α_2-adrenoceptor antagonist in the brain and periphery. In certain preparations idazoxan has some partial agonist activity at α_1 and α_2-adrenoceptors (Hannah *et al.*, 1983; Goldstein *et al.*, 1983; Limberger & Starke, 1983); but in contrast to yohimbine and rauwolscine the other α_2-adrenoceptor antagonists available, it has little effect at receptors other than α-adrenoceptors, and in most cases has greater α_2-selectivity (Doxey *et al.*, 1983). Idazoxan antagonizes the centrally mediated effects of clonidine in both experimental animals and man, indicating that this compound passes the blood-brain barrier after systemic administration (Berridge *et al.*, 1982; Dabire *et al.*, 1981; Clifford *et al.*, 1982). In the absence of a specific α_2-adrenoceptor antagonist which does not cross the blood-brain barrier, we have attempted in the present study to assess separately the central and peripheral effects of clonidine by giving idazoxan either intravenously (i.v.) or intracerebroventricularly (i.c.v.) before the administration of i.v.clonidine. It was anticipated that i.v. idazoxan would antagonize both the peripheral and central actions of clonidine, whereas i.c.v. idazoxan

would not fully inhibit the clonidine-induced reduction of plasma noradrenaline concentration if this fall were in part peripherally mediated. The experiments were performed in anaesthetized rats since we have previously found the reduction in plasma noradrenaline concentration by clonidine to be larger and more reproducible than in conscious rats.

Various studies have shown that systemic administration of α-adrenoceptor agonists such as clonidine stimulates growth hormone secretion in rats through an action at central α-adrenoceptors (Day & Willoughby, 1980; Eden *et al.*, 1981; Eriksson *et al.*, 1982). We have therefore used plasma growth hormone levels as an index of central α-adrenoceptor stimulation in conjunction with plasma noradrenaline as an approximate index of peripheral sympathetic nervous activity.

Methods

Animals and general procedures

Male Wistar normotensive rats (250–350 g, Charles River, U.K.) were anaesthetized with Inactin (sodium salt of 5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid) 100 mg kg^{-1} i.p., an anaesthetic without any hypotensive action (Munoz-Ramirez *et al.*, 1978). The trachea was cannulated and polyethylene catheters (Portex PE50) were placed in the right external jugular vein and ipsilateral common carotid artery. The arterial catheter was advanced so that the tip lay in the aortic arch, and arterial blood pressure was recorded via a Statham P23 ID or Bell and Howell type 4-422 pressure transducer attached to a Grass model 79D poly graph. Blood pressure was recorded continuously. Heart rate was calculated directly from the blood pressure recording every 5 min. Body temperature was maintained by placing the animals on a thermostatically controlled heating pad ('Thermega', Remploy U.K.) set at 38°C.

Administration of drugs

Idazoxan (RX781094) or vehicle (saline) were administered i.v. in a volume of 1 ml kg^{-1} as a 5 min infusion. I.c.v. administration of idazoxan was made via a 30G stainless steel needle attached to a PE10 catheter and a Hamilton microsyringe. A bore hole was drilled in the skull 1.7 mm lateral and 1 mm posterior to bregma. Injections were made in a volume of 10 μl given over 5 min at a depth of 5–5.5 mm from the dorsal surface of the skull. Control animals received 10 μl of saline. Verification of i.c.v. injection sites was made at the end of the experiment by injecting 10 μl of Evans Blue dye and examination of the brain for staining of the ventricular spaces. Clonidine 5 μg kg^{-1} i.v. was administered 5 min after the end of the i.v. or i.c.v. infusion of idazoxan or saline.

Determination of plasma levels of noradrenaline and adrenaline

Blood samples (0.35 ml) were withdrawn from the carotid arterial cannula 5 min before and immediately after idazoxan or vehicle and 5, 10, 30 and 60 min after clonidine 5 μg kg^{-1} i.v. Blood was replaced with an equal volume of saline. The blood samples were centrifuged at 4°C for 15 min, the plasma immediately separated and stored at −80°C until assayed. Plasma concentrations of noradrenaline and adrenaline were determined in duplicate for each sample by a double isotope enzymatic assay (Brown & Jenner, 1981).

In a separate experiment, blood samples were taken 15 min before and immediately after idazoxan 50 μg i.c.v., and 5, 10, 30 and 60 min after saline (1 ml kg^{-1} i.v.) to determine the effect of i.c.v. idazoxan on plasma catecholamines in the absence of clonidine.

In a further control experiment, blood samples were collected from two groups of 5 rats receiving either i.v. or i.c.v. saline in place of idazoxan, and saline 1 mg kg^{-1} i.v. in place of clonidine. Blood samples were taken at the same times and to the same volume as in the above experiments in order to exclude blood loss as a cause of changes obtained in plasma catecholamines.

Determination of plasma levels of growth hormone

Plasma levels of rat growth hormone were measured by double antibody radioimmunoassay using reagents supplied by the National Institute of Arthritis Metabolism and Digestive Diseases (Bethesda, Maryland, USA). Purified rat growth hormone (NIAMDD rat GH-1-4) was iodinated with ^{125}I using the chloramine-T method (Greenwood *et al.*, 1963). Specific antibodies against rat growth hormone (NIAMDD anti-rat GHS-4) were used at a final dilution of 1:6000 giving 40–50% binding when incubated with the labelled hormone for 24 h at 4°C. The detection limit for the assay was 5 ng ml^{-1} of rat growth hormone reference preparation (NIAMMD rat GH-RP-1). Blood samples (0.4 ml) for determination of plasma growth hormone levels were withdrawn from the carotid arterial cannula immediately after idazoxan or vehicle and 5 and 10 min after clonidine 5 μg kg^{-1} i.v. In the control experiment to determine the effects of idazoxan 50 μg i.c.v. alone on plasma growth hormone levels, an additional blood sample was taken 5 min before the administration of

idazoxan. After centrifugation of the samples, the plasma was separated and stored at −20°C until assayed.

Drugs

Inactin (5-ethyl-5-(1-methylpropyl)-2-thiobarbitone sodium) was a gift from Dr Pittman, BYK Ltd, West Germany. Clonidine hydrochloride was obtained from Boehringer Ingelheim, W. Germany. Idazoxan, (2-[2-(1,4-benzodioxanyl)]-2-imidazoline hydrochloride, RX781094) was a gift from Dr J.C. Doxey, Reckitt & Colman, Hull. Inactin was dissolved in sterile water for injection (Kirby-Warwick). Clonidine HCl and idazoxan were dissolved in 0.9% w/v saline. All doses used in the text refer to the respective salts.

Statistical analysis

Results are expressed as mean ± s.e.mean where n refers to the number of observations. One way analysis of variance (ONEWAY) was used to test for significance of responses to drug treatment within experimental groups. Two way analysis of variance (ANOVA) was used to estimate significant differences due to treatments. Significance of differences in the initial pressor response to clonidine were assessed using Student's unpaired t test. All analyses were performed using the 'Minitab' statistical computing system (for details see Ryan *et al.*, 1976).

Results

Clonidine hypotension and bradycardia

In all control animals clonidine 5 μg kg^{-1} i.v. caused the anticipated large falls in mean arterial pressure (30–40 mmHg) and heart rate (40–50 beats min^{-1}): Figures 1 and 2. Pretreatment with idazoxan 300 μg kg^{-1} i.v. caused greater than 90% inhibition of clonidine hypotension 5 and 10 min after dosing. The antagonism of the bradycardiac response to clonidine by idazoxan was approximately 50% at these times.

Idazoxan 10 μg i.c.v., caused slightly less inhibition of clonidine hypotension than 300 μg kg^{-1} i.v. and only a small but significant attenuation of the bradycardia (Figure 2). Idazoxan, 50 μg i.c.v., produced little further inhibition of the hypotension but inhibition of the bradycardia was enhanced ($P < 0.025$, Figure 2).

The initial pressor responses to clonidine 5 μg kg^{-1} i.v. were 34.8 ± 3.5 mmHg and 27.7 ± 3.2 mmHg (n = 10 in each case) in the control groups receiving i.v. and i.c.v. saline. Idazoxan 300 μg kg^{-1} i.v. and 50 μg i.c.v. caused significant attenuation of the pressor response, the rises in mean arterial pressure being 13.6 ± 1.9 mmHg ($P < 0.001$ unpaired t test) and 18.9 ± 1.4 mmHg ($P < 0.05$) respectively. Idazoxan 10 μg i.c.v. had no significant effect on the initial pressor response to clonidine (Δ MAP 22.9 ± 1.34 mmHg).

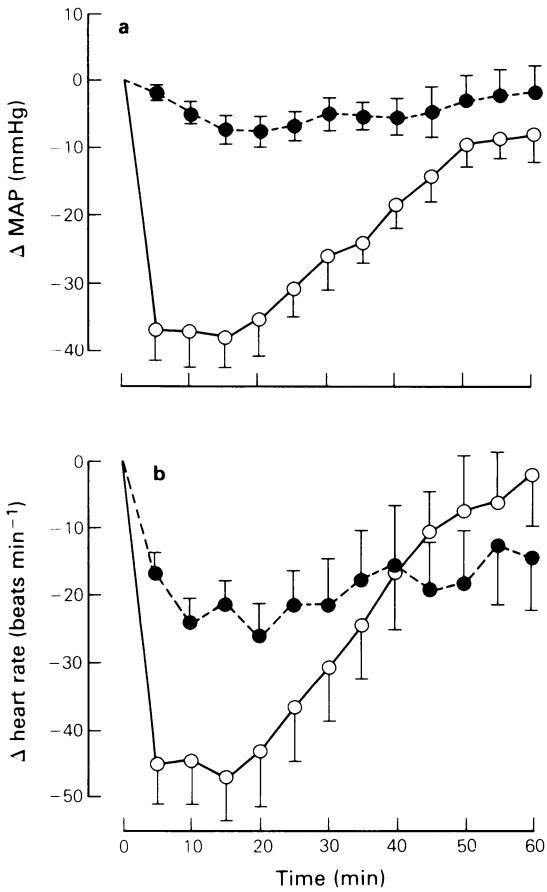

Figure 1 Effects of idazoxan, 300 μg kg^{-1} i.v. (●) or vehicle (saline ○) pretreatment on the decreases in mean arterial pressure (ΔMAP) and heart rate produced by clonidine 5 μg kg^{-1} i.v. Clonidine was given at $t = 0$ min, 5 min after the end of the 5 min infusion of idazoxan or vehicle. Values are the mean ± s.e.mean of 10 observations. Mean arterial pressure prior to clonidine administration was 115.6 ± 6.6 mmHg and 108 ± 3.6 mmHg in the control and idazoxan pretreated groups respectively. Corresponding values for heart rate were 398.4 ± 10.8 and 439.2 ± 7.6 beats min^{-1}.

Plasma catecholamines

In control animals the clonidine-induced hypotension was associated with large reductions in plasma

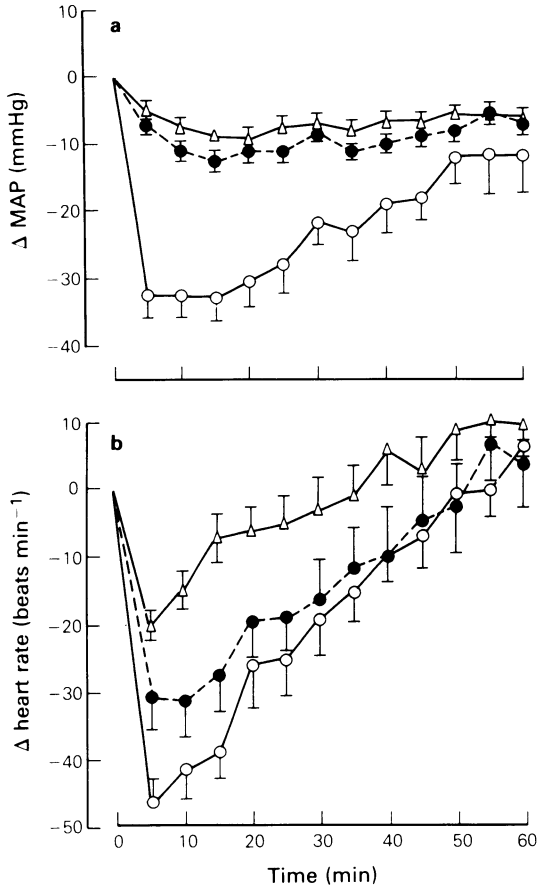

Figure 2 Effects of idazoxan, 10 μg i.c.v. (●) and 50 μg (△), or vehicle (○) pretreatment on the decreases in mean arterial pressure (ΔMAP) and heart rate produced by clonidine 5 μg kg^{-1} i.v. Clonidine was given at $t = 0$ min, 5 min after the end of the 5 min infusion of idazoxan or vehicle (2 μl min^{-1}). Values are the mean ± s.e.mean of 10 observations. Mean arterial pressure prior to clonidine administration was 113.5 ± 4.3 mmHg, 119.5 ± 3.8 mmHg and 115.8 ± 5.9 mmHg in the vehicle, idazoxan 10 μg and idazoxan 50 μg treated groups respectively. Corresponding values for heart rate were 424.2 ± 13.2, 407.4 ± 7.3 and 405 ± 14.4 beats min^{-1} respectively.

catecholamines (Figure 3, Table 1). Idazoxan 300 μg kg^{-1} i.v. caused an immediate rise in plasma noradrenaline to 177.3 ± 9.1% of the control level and almost completely abolished the fall occurring 5 min after clonidine (Figure 3). This rise in plasma noradrenaline was accompanied by an increase in heart rate of 49.8 ± 3.5 beats min^{-1} ($P < 0.001$, $n = 10$) but there were no significant changes in mean arterial pressure during the 5 min infusion of idazoxan ($F_{2,29} = 1.62$, $P > 0.05$ ONEWAY).

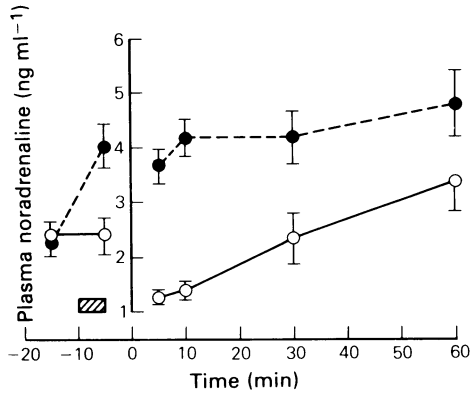

Figure 3 The effect of idazoxan 300 μg kg^{-1} i.v. (●) or vehicle (○) pretreatment on the plasma noradrenaline response to clonidine 5 μg kg^{-1} i.v. Idazoxan or vehicle were infused over 5 min (hatched bar); clonidine (5 μg kg^{-1} i.v.) was given at $t = 0$ min. Values are the mean of 10 observations; s.e.mean shown by vertical lines. Significance value for pretreatment with 300 μg kg^{-1} idazoxan i.v. compared with vehicle pretreatment, $F_{1,108} = 57.1$, $P < 0.001$ (ANOVA).

In control animals receiving 10 μl saline i.c.v. the plasma noradrenaline response to i.v. clonidine was similar to that of the intravenous control group (Figure 4). Pretreatment with 10 μg idazoxan i.c.v. caused only a slight non significant rise in plasma noradrenaline to 118.5 ± 8.6% of control levels with a residual fall of 17.4 ± 2.7% 5 min after clonidine

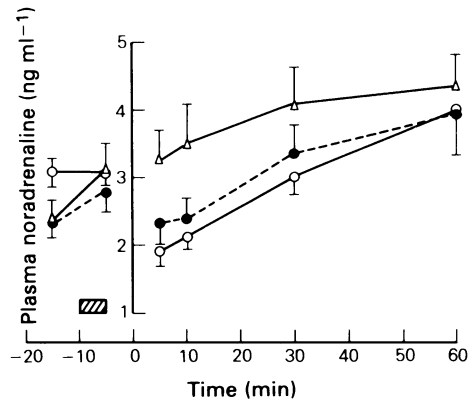

Figure 4 The effect of idazoxan, 10 μg i.c.v. (●) and 50 μg (△) or vehicle (○) pretreatment on the plasma noradrenaline response to clonidine, 5 μg kg^{-1} i.v. Idazoxan or vehicle were infused at 2 μl min^{-1} for 5 min (hatched bar). Clonidine was given at $t = 0$ min. Values are the mean of 10 observations; s.e.mean shown by vertical lines. Significance values compared with vehicle pretreatment 10 μg idazoxan, $F_{1,108} = 0.24$, $P > 0.05$; 50 μg idazoxan $F_{1,108} = 7.23$, $P < = 0.01$ (ANOVA).

Table 1 The effect of central and peripheral administration of idazoxan on the plasma adrenaline responses to clonidine

Experimental group	*Time* (min) −15	−5	+5	+10	+30	+60
Control saline i.v.	0.06 ± 0.01	0.053 ± 0.017	0.035 ± 0.012	0.065 ± 0.03	0.138 ± 0.05	0.140 ± 0.059
Idazoxan 300 μg kg^{-1} i.v.	0.033 ± 0.01	0.056 ± 0.02	0.063 ± 0.02	0.073 ± 0.027	0.083 ± 0.046	0.150 ± 0.04
Control saline i.c.v.	0.043 ± 0.01	0.044 ± 0.01	0.03 ± 0.01	0.046 ± 0.02	0.06 ± 0.014	0.085 ± 0.02
Idazoxan 10 μg i.c.v.	0.035 ± 0.01	0.046 ± 0.01	0.034 ± 0.01	0.055 ± 0.02	0.062 ± 0.01	0.103 ± 0.024
Idazoxan 50 μg i.c.v.	0.031 ± 0.005	0.037 ± 0.008	0.044 ± 0.01	0.041 ± 0.01	0.045 ± 0.007	0.06 ± 0.016

Idazoxan or saline were given as a 5 min infusion from −10 to −5 min. Clonidine, 5 μg kg^{-1} i.v. was given at $t = 0$ min. Plasma adrenaline levels are expressed in ng ml^{-1}. Each value is the mean ± s.e.mean of 10 observations.

(Figure 4). The plasma noradrenaline response to clonidine in animals receiving 10 μg idazoxan i.c.v. was not significantly different from that of the control animals ($F_{1,108} = 0.24$ ANOVA).

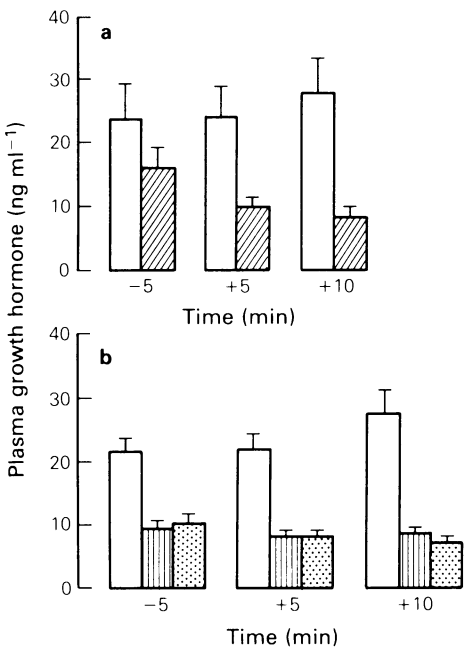

Figure 5 The effect of i.v. (a) and i.c.v. (b) idazoxan on plasma growth hormone in anaesthetized rats. Idazoxan or vehicle were given as a 5 min infusion from −10 to −5 min. Clonidine 5 μg kg^{-1} was given i.v. at $t = 0$ min. (□) vehicle pretreatment; (▨) idazoxan 300 μg kg^{-1} i.v. (⊡) idazoxan 10 μg i.c.v.; (▥) idazoxan 50 μg i.c.v. Results are expressed as ng ml^{-1} NIAMDD rat GH-RP-1. Vertical lines represent the s.e.mean of 10 observations. $P < 0.001$ (ANOVA) for each antagonist dose compared with the respective vehicle pretreatment.

The immediate rise in plasma noradrenaline after idazoxan 50 μg was greater than after the 10 μg dose (130 ± 9.4% of control levels, $P < 0.01$, Figure 4) and was not reversed by the subsequent administration of clonidine.

The plasma adrenaline concentrations at each time point and in each experimental group are shown in Table 1. In both sets of control animals, plasma adrenaline fell 5 min after ($P < 0.05$ in each case) but not 10 min after intravenous clonidine. Idazoxan 300 μg kg^{-1} i.v. and 50 μg i.c.v. completely abolished clonidine-induced falls in plasma adrenaline. However, a significant fall in plasma adrenaline of 24.6 ± 8.6% ($P < 0.05$) still occurred 5 min after intravenous clonidine in animals pretreated with 10 μg idazoxan i.c.v. (Table 1).

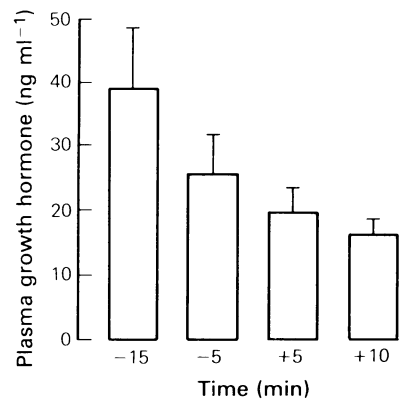

Figure 6 Reduction of plasma growth hormone concentration by idazoxan 50 μg i.c.v. in anaesthetized rats. Idazoxan was infused i.c.v. at 2 μl min^{-1} from −10 to −5 min. Saline (1 ml kg^{-1} i.v.) was given at $t = 0$ min. Vertical lines represent the s.e.mean of 8 observations; $P < 0.05$ (ONEWAY).

Table 2 The effect of intracerebroventricular idazoxan (50 μg) on plasma noradrenaline and adrenaline

	Time (min)							
	−30	−15	−5	+5	+10	+30	+60	*P* value (ONEWAY)
Noradrenaline ng ml^{-1}	0.22 ± 0.016	0.22 ± 0.018	0.30 ± 0.03	0.34 ± 0.04	0.37 ± 0.04	0.41 ± 0.05	0.44 ± 0.05	$P < 0.001$
Adrenaline ng ml^{-1}	0.033 ± 0.01	0.036 ± 0.01	0.036 ± 0.01	0.036 ± 0.01	0.054 ± 0.01	0.09 ± 0.02	0.1 ± 0.02	$P < 0.005$

Idazoxan (50 μg i.c.v.) was given as a 5 min infusion from −10 to −5 min. Saline (1 ml kg^{-1} i.v.) was given at $t = 0$ min. Each value is the mean ± s.e.mean of 8 observations.

In a separate group of animals receiving 50 μg idazoxan i.c.v. and saline 1 ml kg^{-1} i.v. in place of clonidine, both noradrenaline and adrenaline rose slowly and significantly over the period of the experiment (Table 2). The rise in plasma catecholamines was unaccompanied by any significant effects on mean arterial pressure and heart rate. The plasma noradrenaline response in this group of animals was similar to that of animals pretreated with 50 μg idazoxan i.c.v. and given clonidine 5 μg kg^{-1} i.v. (Figure 4).

Repeated blood sampling from animals given saline alone in place of both idazoxan and clonidine produced slight but non-significant elevation of both plasma noradrenaline and adrenaline. Plasma noradrenaline rose from 0.253 ± 0.048 ng ml^{-1} at − 15 min to 0.313 ± 0.086 ng ml^{-1} at 60 min, and from 0.357 ± 0.045 ng ml^{-1} to 0.454 ± 0.05 ng ml^{-1} in i.v. and i.c.v. saline-treated animals which received no clonidine ($n = 5$ in each case, $P > 0.05$ ONEWAY). Similarly, plasma adrenaline rose from 0.025 ± 0.006 ng ml^{-1} to 0.037 ± 0.01 ng ml^{-1} and from 0.037 ± 0.006 ng ml^{-1} to 0.039 ± 0.005 ng ml^{-1} in the i.v. and i.c.v. saline pretreated groups respectively.

Plasma growth hormone

All studies were performed in rats anaesthetized with barbiturate and high levels of plasma growth hormone were found in control animals (Figure 5). No significant rise in plasma growth hormone was seen in these rats 5 or 10 min after i.v. clonidine. However, pretreatment with both i.v. and i.c.v. idazoxan resulted in suppression of growth hormone secretion compared with the respective controls (Figure 5). Idazoxan 50 μg i.c.v. produced no further suppression of growth hormone secretion compared with idazoxan 10 μg i.c.v. (Figure 5b). In the control group of rats which received 50 μg idazoxan i.c.v. and saline 1 ml kg^{-1} i.v. in place of clonidine, there was a gradual reduction of plasma growth hormone levels after the idazoxan administration (Figure 6).

Discussion

Idazoxan has been used recently in both man and experimental animals as an α-adrenoceptor antagonist with greater α_2:α_1 selectivity than yohimbine (Doxey *et al.*, 1983; Clifford *et al.*, 1982; Caroon *et al.*, 1982). In this study Idazoxan almost fully antagonized the reduction in mean arterial pressure and to a lesser extent, the reductions in heart rate and plasma catecholamines produced by i.v. clonidine. The question is whether the study supports the hypothesis that there is a peripheral component to

the sympatholytic action of clonidine, and provides evidence for a role of peripheral α_2-adrenoceptors in the control of noradrenaline release. These questions have arisen because of the lack of a specific α_2-adrenoceptor antagonist which does not cross the blood-brain barrier. The present study was therefore designed to use the indirect approach of comparing the effects of clonidine after central or peripheral administration of the α_2-adrenoceptor antagonist, idazoxan. The most striking difference appeared to be the effect of the antagonist itself on plasma noradrenaline concentration, before administration of clonidine. Almost two fold increases in plasma noradrenaline were noted after i.v. idazoxan, more than four fold greater than after the 10 μg i.c.v. dose. The second difference between the i.v. and 10 μg i.c.v. doses was the lack of significant inhibition by the latter of the clonidine-induced fall in plasma noradrenaline concentration (Figure 4). These differences suggest that both stimulation and antagonism of peripheral α_2-adrenoceptors can modulate sympathetic nerve release of noradrenaline *in vivo*, as reflected by plasma noradrenaline concentration. However, this argument assumes that the two doses of idazoxan 300 $\mu g\,kg^{-1}$ i.v. and 10 μg i.c.v. provided a similar degree of blockade of central α_2-adrenoceptors, and the evidence for this should be considered.

Our original intention was to use plasma growth hormone as a marker of central α-adrenoceptor stimulation. Previous studies have shown that systemic administration of α-adrenoceptor agonists such as clonidine, which pass the blood-brain barrier, stimulates growth hormone secretion in both previously untreated rats and rats pretreated with reserpine (Day & Willoughby, 1980; Eden *et al.*, 1981; Eriksson *et al.*, 1982). The effects of clonidine can be prevented by pretreatment with the α_2-adrenoceptor antagonist yohimbine but not by phenoxybenzamine (Eriksson *et al.*, 1982). However, we underestimated the effect of barbiturate anaesthesia itself as a stimulus to growth hormone release, and little further increase was seen after clonidine administration in our experiments. Nevertheless, idazoxan antagonized the barbiturate-induced increases, and this antagonism was as great after 10 μg i.c.v. as after either the i.v. dose or the 50 μg i.c.v. dose. This suggests that the 10 μg icv dose was sufficient to achieve a near maximal degree of blockade at central α_2-adrenoceptors. A further argument to support this hypothesis is that the higher i.c.v. dose of idazoxan did not achieve any greater blockade of the clonidine-induced hypotension; it is likely that the 10 μg i.c.v. dose already blocked the α_2-adrenoceptor mediated component in the hypotension, and that the residual effect of clonidine was due to its α_1-adrenoceptor agonist activity. The explanation for the greater inhibition by idazoxan 50 μg i.c.v. (than 10 μg) of clonidine induced falls in plasma catecholamines is probably that the 50 μg dose causes some degree of peripheral α_2-adrenoceptor blockade. Considering the weight of the animals used in this study, 50 μg i.c.v. is approximately 150–200 $\mu g\,kg^{-1}$, one half to two thirds of the dose administered i.v. This dose of idazoxan also partially antagonized the initial pressor effect of clonidine. We attempted to quantify the amount of idazoxan which diffused out of the brain, but plasma levels of idazoxan were largely below the quantitation limit (10 $ng\,ml^{-1}$) of the available h.p.l.c. assay after i.c.v. administration of 50 μg (results not shown). Following i.v. administration in the rat, peak plasma levels of idazoxan obviously are much higher than after i.c.v. dosing, for instance 115 $ng\,ml^{-1}$, less than 5 min after a dose of 0.5 $mg\,kg^{-1}$ (Clifford *et al.*, 1983). However, plasma levels decline rapidly as idazoxan distributes into tissues, much higher levels being found in brain, liver, kidney and lung. Levels of unchanged idazoxan in brain were 6–13 fold higher than for plasma (Clifford *et al.*, 1983). Using [^{3}H]-idazoxan we have found that 5 min after the end of a 5 min i.v. infusion of 300 $\mu g\,kg^{-1}$ idazoxan, plasma levels of radioactivity are little higher than observed after i.c.v. administration of the same dose ($n=6$, data not shown).

Idazoxan was chosen as the probe in the present experiments as it is the most specific α_2-adrenoceptor antagonist available. I.c.v. injection of other α-adrenoceptor antagonists which are more polar than idazoxan and therefore would not cross the blood brain barrier so rapidly, may in theory be expected not to inhibit a peripherally-induced fall in plasma noradrenaline after i.v. clonidine. However, high doses of the non-selective α-adrenoceptor antagonist phentolamine must be used i.c.v. to inhibit the hypotensive effect of clonidine (Finch, 1974; Finch *et al.*, 1975). Such high concentrations of phentolamine may have additional effects such as inhibition of neuronal uptake of noradrenaline. Berridge *et al.* (1982) were unable to block the hypotensive effect of i.v. clonidine in anaesthetized rats with the selective α_2-adrenoceptor antagonists yohimbine and rauwolscine given i.c.v. in fairly high doses. These authors concluded that of the α_2-adrenoceptor antagonists studied, only idazoxan was potent and soluble enough after i.c.v. administration to inhibit clonidine.

Some reduction in plasma noradrenaline concentration may occur as a result of stimulation of the sympathetic component of the baroreceptor reflex by the initial pressor response to clonidine. This increase in blood pressure is transient and the hypotensive response to clonidine predominates within 1 min of i.v. administration. In the present study blood samples for the determination of plasma catecholamines

were taken 5 and 10 min after i.v. clonidine when the hypotensive response was already maximal. It therefore seems unlikely that baroreceptor-induced reflex changes in sympathetic nerve activity contributed to the falls in plasma noradrenaline concentration observed at these times.

It is interesting that despite our evidence for a peripheral contribution to the sympatholytic effect of clonidine, we confirmed the extensive previous evidence that the hypotensive effect is centrally mediated (Schmitt, 1977; Kobinger, 1978;, Van Zwieten & Timmermans, 1979; Timmermans *et al.*, 1981). This might support the suggestion that clonidine-induced hypotension is not entirely due to its sympatholytic action (Schmitt, 1975; Laubie *et al.*, 1976). However, it might also reflect some inadequacy of measurement of plasma noradrenaline as an index of peripheral sympathetic activity or the insignificance of peripheral prejunctional adrenoceptors in blood pressure control. It is notable that the large increase in plasma noradrenaline following i.v. idazoxan administration was not associated with any elevation of blood pressure.

The concentration of noradrenaline in plasma is the net result of the rate at which noradrenaline diffuses into plasma and the rate at which it is removed from plasma; therefore the possibility must always be considered that drug-induced changes in noradrenaline clearance may alter the plasma noradrenaline level without altering release of noradrenaline from the nerve endings. However, clonidine decreases both plasma noradrenaline and the noradrenaline release rate in anaesthetized rabbits with no effect on clearance except at high doses (Majewski *et al.*, 1982). The effect of idazoxan on noradrenaline clearance has not been directly estimated, but adrenaline clearance in human volunteers was shown to be unaffected by idazoxan (Brown & Dollery, 1984).

Other studies have shown that the α_2-adrenoceptor antagonists yohimbine and rauwolscine increase plasma noradrenaline and the noradrenaline release rate into plasma in both anaesthetized and conscious rabbits (Majewski *et al.*, 1983a,b) However, in contrast to our results with idazoxan, these effects were accompanied by a decrease and an increase in blood pressure respectively, in the two experimental models. In addition, the relatively selective α_2-adrenoceptor agonist α-methylnoradrenaline decreased plasma noradrenaline and the noradrenaline release rate in conscious rabbits (Majewski *et al.*, 1983b). Its effects were inhibited by yohimbine pretreatment and were therefore probably mediated through α_2-adrenoceptors. These results are consistent with a physiological operation of prejunctional α_2-adrenoceptors at peripheral sympathetic nerve terminals.

Hannah *et al.* (1983) found that idazoxan did not antagonize the bradycardia observed after i.v. clonidine in the conscious rabbit, whereas in the anaesthetised dog (under the code name 170-150) and rat idazoxan caused significant attenuation of clonidine bradycardia (Dabire *et al.*, 1981; Berridge *et al.*, 1982). Interpretation of the data in the rabbit is difficult as idazoxan itself caused a long lasting fall in heart rate in this preparation, in contrast to the significant increase in heart rate found in the present study in anaesthetized rats and also in anaesthetized dogs (Dabire *et al.*, 1981). There is, however, some evidence that mechanisms other than α-adrenoceptor activation may be involved in clonidine-induced bradycardia (Elliot *et al.*, 1981). De Jonge *et al.* (1981) have shown that stimulation of cardiac prejunctional α_2-adrenoceptors by clonidine and some of its analogues contributes to the bradycardiac action in anaesthetized rats. This may explain why we have found that idazoxan 10 μg i.v. produced only a slight inhibition of clonidine bradycardia but similar inhibition of the hypotension as 50 μg i.c.v.

In conclusion, the results presented here suggest that stimulation of peripheral prejunctional α_2-adrenoceptors contributes to the sympatholytic effect of intravenous clonidine and confirm the findings of many previous studies that the hypotensive effect of clonidine is centrally mediated.

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