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A comparative study of the reversal by different α_2-adrenoceptor antagonists of the central sympatho-inhibitory effect of clonidine

Christine Vayssettes-Courchay, Françoise Bouysset, Alex A. Cordi, Michel Laubie & [1]Tony J. Verbeuren

Division of Angiology, Servier Research Institute, 11 rue des Moulineaux, 92150 Suresnes, France

1 The recovery of the clonidine-induced hypotension, bradycardia and sympatho-inhibition produced by several putative α_2-adrenoceptor antagonists was investigated in pentobarbitone anaesthetized rats. The activity of four substances containing an imidazoline structure: idazoxan, methoxy-idazoxan, BRL44408 and atipamezole was compared with the effect of fluparoxan, yohimbine and L-657,743; in addition the effect of the α_1-adrenoceptor antagonist, prazosin, was also studied.

2 Prazosin (0.03–1 mg kg^{-1}, i.v.) failed to alter the sympatho-inhibitory and hypotensive effects of clonidine (10 μg kg^{-1}, i.v.). L-657,743 (0.01–1 mg kg^{-1}, i.v.) induced a recovery of blood pressure, heart rate and renal sympathetic nerve activity. Yohimbine (0.03–3 mg kg^{-1}, i.v.) completely reversed the sympatho-inhibitory effect of clonidine but did not alter its hypotensive effect.

3 The four imidazoline drugs: idazoxan (10–300 μg kg^{-1}, i.v.), methoxy-idazoxan (1–100 μg kg^{-1}, i.v.), BRL44408 (0.1–3 mg kg^{-1}, i.v.) and atipamezole (0.03–1 mg kg^{-1}, i.v.) and fluparoxan (10–300 μg kg^{-1}, i.v.) reversed the clonidine-induced hypotension but produced only a partial recovery of the renal sympathetic nerve activity and of the heart rate. After pretreatment with prazosin (0.1 mg kg^{-1}, i.v.), the recovery of the sympathetic nerve activity elicited by these compounds was significantly higher. In hexamethonium (10 mg kg^{-1}, i.v.) pretreated rats, these five drugs induced dose-related hypertension which was reduced by pretreatment with prazosin (0.1 mg kg^{-1}, i.v.).

4 Our results indicate that the putative α_2-adrenoceptor antagonists idazoxan, methoxy-idazoxan, BRL44408, atipamezole and fluparoxan also have a peripheral hypertensive effect which is mediated through activation of vascular α_1-adrenoceptors; this property of the compounds may be partly responsible for the reversal of the hypotensive action of clonidine. Considering the structure and the affinities of the drugs tested, our data indirectly suggest that α_{2A}-adrenoceptors may be implicated in the central sympatho-inhibitory effects of clonidine.

Keywords: Clonidine; α-adrenoceptor; blood pressure; sympathetic nerve activity

Introduction

Clonidine, when administered intravenously, induces biphasic effects on blood pressure: a transient hypertension followed by a persistent decrease in blood pressure. It is well established that the delayed response to i.v. clonidine is due to a central effect which decreases arterial blood pressure and heart rate via an inhibition of sympathetic nerve activity (Schmitt *et al.*, 1973). This action has been localized at the level of the medulla (see, Laubie *et al.*, 1976) and more precisely the site of action has been demonstrated to be located in the rostral ventrolateral medulla (Bousquet *et al.*, 1981; Sun & Guyenet, 1986). α_2-Adrenoceptors have been implicated in this central action of clonidine (Schmitt, 1977; Timmermans *et al.*, 1981). At the same time it has been suggested that another receptor, not an adrenoceptor, could be involved in some of the central effects of clonidine (see Karppanen, 1981) and later, from the observation that other drugs with an imidazoline ring induce hypotension when microinjected into the rostral ventrolateral medulla, it has been suggested that clonidine could also act on an imidazoline-binding-site to produce sympatho-inhibition (Bousquet *et al.*, 1984; Ernsberger *et al.*, 1990). Different types of imidazoline-binding sites, also named idazoxan-binding sites (Wikberg, 1989) were suggested (Michel & Insel, 1989; Brown *et al.*, 1990). The existence of two different types was shown (Wikberg *et al.*, 1991), named I_1 and I_2 (Michel & Ernsberger, 1992). Clonidine binds to both α_2-adrenoceptors and imidazoline-sites in the brain (Ernsberger *et al.*, 1988; Brown *et al.*, 1990). The putative role of imidazoline binding sites in the action of clonidine was supported by the evidence that they are present in the ventrolateral medulla (Ernsberger *et al.*, 1987); these sites belong to the I_1 subtype and clonidine binds preferentially to this subtype (Wikberg *et al.*, 1991). However, recent studies indicate that the decreases in sympathetic nerve activity and blood pressure caused by clonidine implicate mainly α_2-adrenoceptors in the conscious rabbit (Head *et al.*, 1993) as well as in the anaesthetized rat (Hieble & Kolpak, 1993).

The goal of the present study was to analyse the effects of different α_2-adrenoceptor antagonists on the central sympatho-inhibitory effect of clonidine. The α_2-adrenoceptors have been classified into four major subtypes, α_{2A}, α_{2B}, α_{2C} and α_{2D} (Bylund, 1985; Nahorski *et al.*, 1985; Bylund *et al.*, 1991). The α_{2A} and the α_{2D} adrenoceptors appear to be the same subtype with some different pharmacological properties depending on the species (Link *et al.*, 1992; see Bylund *et al.*, 1995). We compared the activity of antagonists for which different affinities to α_1, α_{2A}, α_{2B}-adrenoceptors and imidazoline-binding-sites have been reported. Four imidazoline drugs: idazoxan, methoxy-idazoxan, BRL44408, atipamezole and four non-imidazoline drugs: yohimbine, fluparoxan, L-657,743, prazosin (Figure 1) were investigated. Idazoxan was used because of its affinity for both α_2-adrenoceptors and imidazoline-binding-sites (Brown *et al.*, 1990); the compound has a better affinity for the I_2 subtype (Wikberg *et al.*, 1991). Methoxy-idazoxan is a specific α_{2A}-adrenoceptor antagonist (Langin *et al.*, 1989; Hudson & Nutt, 1990). Atipamezole, which does not show selectivity toward

[1] Author for correspondence

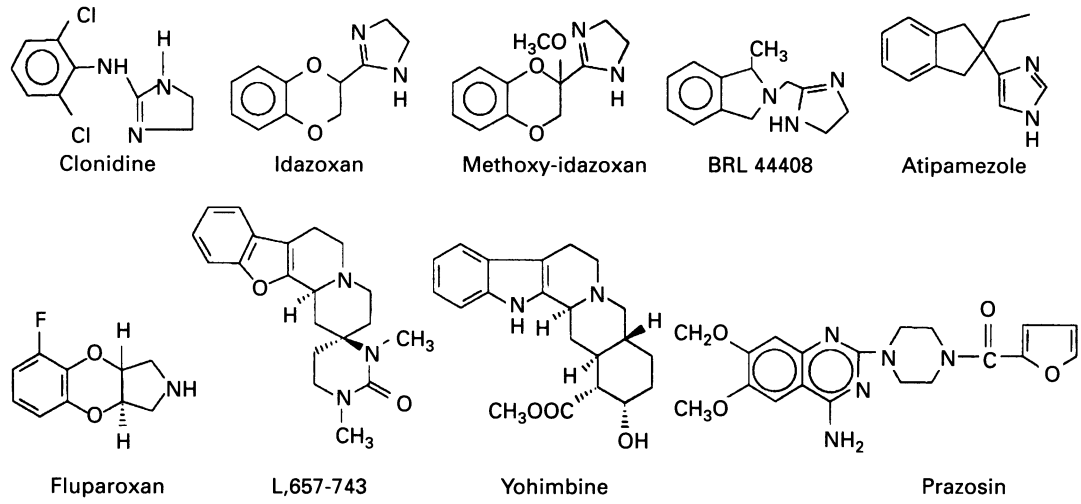

Figure 1 Chemical structure of the α-adrenoceptor agents used.

one of the α_2-adrenoceptor subtypes (Blaxall *et al.*, 1991), and BRL44408 are α_2-adrenoceptor antagonists and were chosen because of their imidazoline structure. Fluparoxan and L-657,743 are α_2-adrenoceptor antagonists without imidazoline structure. Yohimbine has been used frequently as an α_{2A} and α_{2B}-adrenoceptor antagonist (Brown *et al.*, 1990) because of its different affinities as compared to idazoxan (Hamilton *et al.*, 1988), and this in spite of its affinity for α_1-adrenoceptors and 5-HT receptors. Lastly prazosin is an α_1-adrenoceptor antagonist which also binds to α_{2B}-adrenoceptors (Bylund *et al.*, 1988).

A preliminary report of some of the data has been presented at the meeting *Pharmacology of Adrenoceptors*, King of Prussia, U.S.A., July 1994.

Methods

Male Sprague Dawley rats weighing 350–430 g were anaesthetized with sodium pentobarbitone: 50 mg kg^{-1}, i.p. was the initial dose followed by a continuous infusion at 12 mg kg^{-1} h^{-1}. Artificial ventilation was performed with a Harvard rodent ventilator and body temperature was maintained at 38°C with an homeothermic blanket. Systemic arterial blood pressure was measured from the femoral artery via a Statham P10EZ transducer connected to a pressure transducer (Gould) and the heart rate was measured with a tachograph (Gould) triggered by the pressure pulse. A femoral vein was cannulated for the intravenous administration of drugs. Data were recorded and analysed as previously described (Vayssettes-Courchay *et al.*, 1990; 1993). The left pre-renal nerve was exposed by a retroperitoneal approach, dissected free and placed on a bipolar stainless steel electrode (diameter 0.08 mm); the nerve was isolated and preserved with a bath of fluorinert (Sigma). The nerve signal was amplified (DAM 60 WPI) with a band pass of 300 Hz–1 kHz and measured (μV s^{-1}) with a Gould integrator. The level of noise was recorded at the end of the experiment after application of xylocaine (5%) to the nerve. The control value of renal nerve activity was defined as 100% after subtraction of the noise. The arterial blood pressure, heart rate and renal nerve activity were recorded on a magnetic tape and displayed on a Gould ES1000 recorder. The data were analysed with a Compaq 386s computer coupled to a CED 1401 laboratory interface and a SPIKE2 software (CED) and they are shown as mean ± s.e.mean. The arterial blood pressure was expressed in mm mercury (mmHg), the heart rate in beats min^{-1} and the renal nerve activity in %. When the reversal of the effects of clonidine was studied, dose-response curves as % recovery were constructed and the maximal effects observed were determined; IC_{50} values were obtained by computer non linear regression using the Simplex method (Caceci & Cacheris, 1984), calculated with the equation of Michaelis & Menten: $E = (E_{max} * C^n)/(EC^n + C^n)$. When the hypertensive effect of the drugs was studied, the dose-response curves in mmHg were constructed and the maximal hypertension observed were determined; EC_{50} values were obtained with the method described above. Student's *t* test for paired and unpaired observations was used to assess the statistical significance of the results.

After a stabilization period, the control values were determined and clonidine was injected. Antagonists were administered in increasing doses with 4 min intervals, 10 min after clonidine administration. In some groups of rats, prazosin was administered 10 min before clonidine. Five rats were used in each group. In some experiments, the rats were pretreated with i.v. hexamethonium (10 mg kg^{-1}, 10 min before further injections) to produce ganglionic blockade; in these animals, the renal nerve activity was not recorded.

The drugs used were: idazoxan hydrochloride, (RBI); yohimbine hydrochloride, (Sigma); fluparoxan hydrochloride (GR50360, Glaxo); prazosin hydrochloride, (Sigma); L-657,743 hydrochloride (gift: Dr Clineschmidt, Merck); methoxy-idazoxan free base (RX821002), BRL44408 free base, atipamezole hydrochloride and clonidine hydrochloride were synthesized by Dr Cordi (Servier Research Institute). The quantities used refer to the free bases. Idazoxan, fluparoxan, L-657,743, atipamezole and clonidine were dissolved in saline; yohimbine and prazosin were dissolved in isotonic glucose solution; methoxy-idazoxan was dissolved in 5% HCl 0.1N, 94% saline and 1% NaOH 0.1N (pH 7.5); BRL44408 was dissolved in 11% HCl 0.1N, 89% glucose solution and 0.2% NaOH 0.1N (pH 7.35).

Results

Reversal of the central action of clonidine

Control rats For eight groups of five rats the control values of mean blood pressure, heart rate and renal nerve activity (MBP, HR and RNA) are shown in Table 1. Clonidine (10 μg kg^{-1}, i.v.) caused an immediate transient hypertensive effect in the different groups of animals which varied between +48±7 to +58±3 mmHg and was associated with a decrease in heart rate varying from −32±5 to −51±15 beats min^{-1} and a decrease in renal nerve activity varying from −77±9 to −86±4%. Ten minutes after clonidine administration, the

maximal and long-lasting sympatho-inhibitory, hypotensive and bradycardic effects were obtained; these effects did not differ significantly in the different groups of rats, as indicated in Table 1. In each group of rats, one of the eight drugs was then administered i.v. at cumulative doses. Table 1 illustrates the effects of the antagonists, expressed as either the maximal % of recovery of the clonidine responses or as the IC_{50} values in $\mu g\ kg^{-1}$ and in $\mu mol\ kg^{-1}$. The dose-related reversion of the effect of clonidine by each drug is represented in Figure 2.

Prazosin (0.03 to 1 $\mu g\ kg^{-1}$), methoxy-idazoxan (1 to 100 $\mu g\ kg^{-1}$), fluparoxan (10 to 300 $\mu g\ kg^{-1}$), BRL44408 (0.1 to 3 $mg\ kg^{-1}$) and atipamezole (0.003 to 1 $mg\ kg^{-1}$) completely reversed the clonidine-induced hypotension, leading to mean blood pressure values higher than those observed before

Table 1 Effect of adrenoceptor antagonists on mean arterial blood pressure (MBP), heart rate (HR) and renal nerve activity (RNA) after clonidine (Clo) 10 $\mu g/kg^{-1}$, i.v. in the anaesthetized rat[a]

		Idazoxan	*Meth-Idaz*	*BRL-44408*	*Atipamezole*	*Fluparoxan*	*L-657,743*	*Yohimbine*	*Prazosin*
MBP	Before (mmHg)	120 ± 5	117 ± 4	119 ± 6	127 ± 8	118 ± 2	125 ± 6	121 ± 8	116 ± 7
	After Clo	-26 ± 5	-33 ± 6	-22 ± 3	-24 ± 4	-31 ± 6	-26 ± 5	-32 ± 7	-26 ± 5
	max.recovery (%)	108 ± 11[c]	121 ± 12[c]	116 ± 41[c]	157 ± 9[c]	113 ± 19[c]	82 ± 8[c]	14 ± 14	ND
	IC_{50} $\mu g\ kg^{-1}$	24.6	3.9	770.7	11.8	66.9	24.5	21270	ND
	IC_{50} $\mu mol\ kg^{-1}$	0.023	0.017	3.6	0.056	0.28	0.076	60	ND
HR	Before (b.p.m.)	402 ± 6	417 ± 8	404 ± 11	430 ± 8	437 ± 14	429 ± 14	383 ± 13	411 ± 10
	After Clo	-71 ± 5	-85 ± 14	-74 ± 7	-90 ± 16	-99 ± 15	-98 ± 14	-76 ± 12	-86 ± 10
	max.recovery (%)	74 ± 17[c]	112 ± 4[c]	32 ± 9[c]	36 ± 20	86 ± 17[c]	102 ± 17[c]	65 ± 15[c]	ND
	IC_{50} $\mu g\ kg^{-1}$	34.2	7.6	11980	11.2	66.4	24.1	969.6	ND
	IC_{50} $\mu mol\ kg^{-1}$	0.17	0.03	55.6	0.05	0.28	0.075	2.74	ND
RNA	After Clo (%)	-78 ± 5	-80 ± 5	-82 ± 3	-90 ± 2	-82 ± 2	-77 ± 9	-83 ± 5	-83 ± 7
	max.recovery (%)	77 ± 4[c]	76 ± 10[c]	36 ± 8[c]	66 ± 10[c]	58 ± 4[c]	97 ± 17[c]	99 ± 10[c]	5 ± 6
	IC_{50} $\mu g\ kg^{-1}$	17.1	3.6	119.5	7.2	17.3	27.9	579	61.1
	IC_{50} $\mu mol\ kg^{-1}$	0.08	0.015	0.56	0.034	0.072	0.086	1.6	0.16

$n = 5$

[a]The control value before clonidine (before), the effect of clonidine 10 min after its administration (after Clo), the maximal % of recovery, the IC_{50} values in $\mu g\ kg^{-1}$ and the IC_{50} values in $\mu mol\ kg^{-1}$ are indicated (the latter correspond to the doses inducing 50% of the maximal recovery). The control value of RNA before clonidine was taken as 100%. [b]The decrease in MBP, HR and RNA with clonidine were significant in all groups of rats ($P < 0.05$). [c]The recovery is significant ($P < 0.05$).

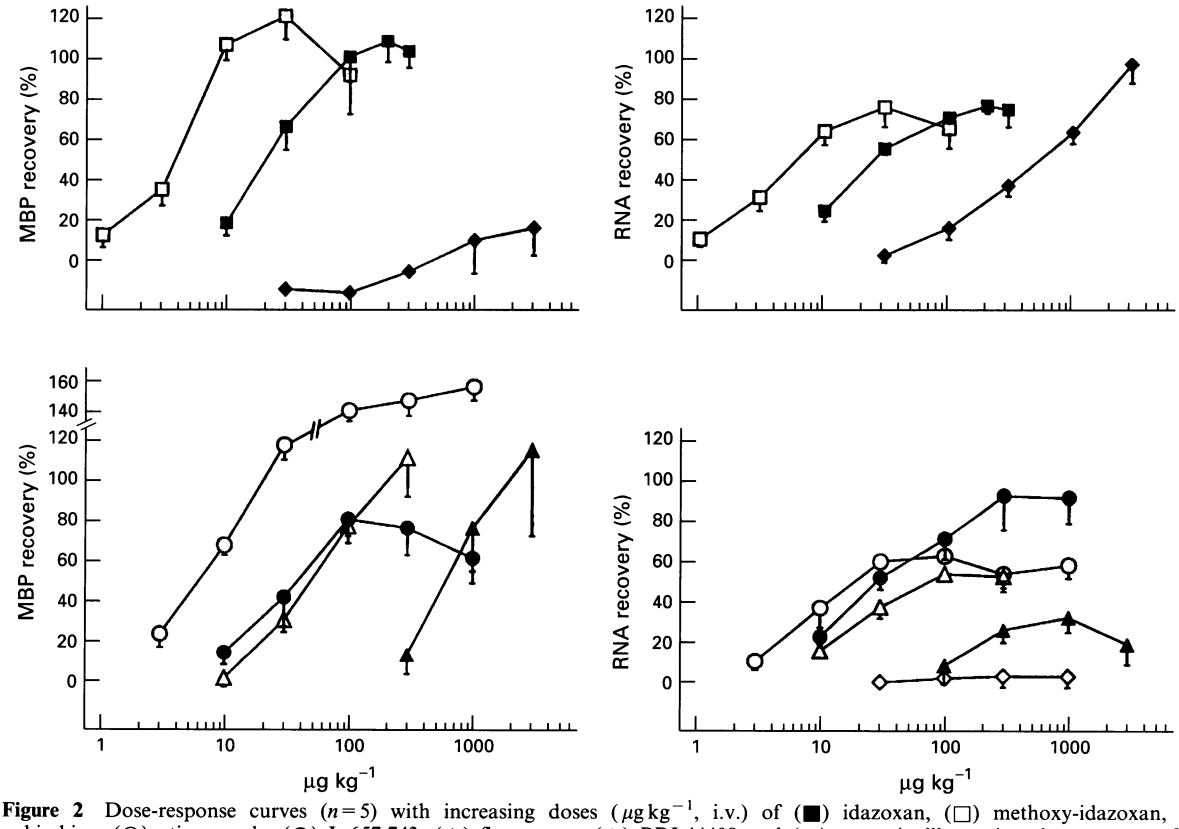

Figure 2 Dose-response curves ($n = 5$) with increasing doses ($\mu g\ kg^{-1}$, i.v.) of (■) idazoxan, (□) methoxy-idazoxan, (◆) yohimbine, (○) atipamezole, (●) L-657,743, (△) fluparoxan, (▲) BRL44408 and (◇) prazosin illustrating the recovery of the effects of clonidine (10 $\mu g\ kg^{-1}$, i.v.) on mean blood pressure (MBP, left panel) and renal sympathetic nerve activity (RNA, right panel).

clonidine administration. These five compounds reversed only partially the sympatho-inhibitory effect of clonidine. Yohimbine (0.03 to 3 mg kg^{-1}) completely reversed the sympatho-inhibition but only weakly reversed the hypotension induced by clonidine. L-657,743 (10 μg to 1 mg kg^{-1}) reversed both the hypotension and the decrease in RNA induced by clonidine (Figure 3). The bradycardia was completely reversed by L-657,743 and methoxy-idazoxan, partially by fluparoxan, idazoxan and yohimbine and only moderately by BRL 44408 and atipamezole. To judge from the IC_{50} values, the order of potency for reversal of the clonidine effects is as follows: for mean blood pressure, methoxy-idazoxan $\geqslant$ idazoxan > atipamezole $\geqslant$ L657,743 > fluparoxan > BRL44408 > yohimbine; for heart rate methoxy-idazoxan $\geqslant$ atipamezole $\geqslant$ L-657,743 > idazoxan $\geqslant$ fluparoxan > yohimbine > BRL44408; for renal nerve activity, methoxy-idazoxan > atipamezole > fluparoxan $\geqslant$ idazoxan $\geqslant$ L-657,743 > BRL44408 > yohimbine.

Prazosin-treated rats Since in normal rats, idazoxan, methoxy-idazoxan, fluparoxan, BRL 44408 and atipamezole caused less reversal of the sympatho-inhibitory effect of clonidine than of its hypotensive effect, the experiments for these five compounds were repeated with the same cumulative doses after blockade of the α_1-adrenoceptors with prazosin. Prazosin (0.1 mg kg^{-1}, i.v.) was administered 10 min before clonidine in five groups of rats. Clonidine and the antagonist were applied as previously described.

As expected, the administration of the α_1-adrenoceptor antagonist, prazosin, decreased blood pressure to between 80 ± 4 and 95 ± 9 mmHg. Consequently both the hypertensive and hypotensive effects of clonidine were altered and the antagonists no longer significantly modified the pressor responses to clonidine.

The bradycardic and sympatho-inhibitory effects of clonidine were not altered in rats pretreated with prazosin. The inhibitory effect of the five antagonists on the sympatho-inhibitory action of clonidine was increased (Table 2), significantly for BRL44408 and fluparoxan. Idazoxan, methoxy-idazoxan, atipamezole and fluparoxan almost completely reversed the clonidine-induced sympatho-inhibition, whereas BRL44408 reversed it by $71 \pm 12\%$ (Figure 4).

Methoxy-idazoxan and atipamezole which only partially reversed the bradycardic effect of clonidine in control rats, completely reversed it in the presence of prazosin; fluparoxan completely reversed it in both cases, whereas the reversal by idazoxan and BRL44408, which were partial in the control rats, were increased and decreased, respectively after prazosin-treatment (Table 2). To judge from the IC_{50} values, the order of potency of the five antagonists was: for heart rate, methoxy-idazoxan > BRL44408 $\geqslant$ atipamezole > idazoxan $\geqslant$ fluparoxan; for renal nerve activity, methoxy-idazoxan > atipamezole > idazoxan $\geqslant$ fluparoxan > BRL44408. This order of potency is comparable to that observed in control rats except for BRL44408 which on the heart rate response was more potent.

Hypertensive effect of the antagonists

Effects after pretreatment with hexamethonium Since the antagonist actions of idazoxan, methoxy-idazoxan, fluparoxan, BRL44408 and atipamezole were different before and after blockade of the α_1-adrenoceptors, the peripheral action of these compounds was investigated after blockade of the sympathetic ganglia with hexamethonium (10 mg kg^{-1}, i.v., administered 10 min before the antagonist).

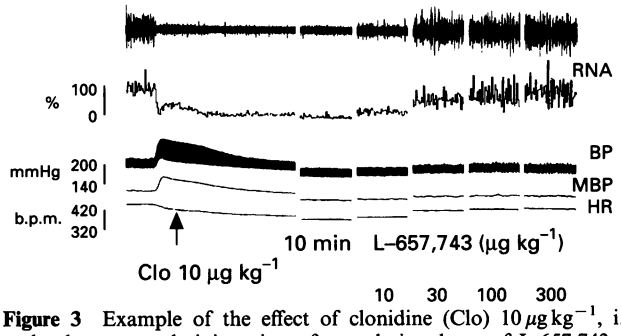

Figure 3 Example of the effect of clonidine (Clo) 10 $\mu g\,kg^{-1}$, i.v. and subsequent administration of cumulative doses of L-657,743 on renal nerve activity (RNA), blood pressure (BP), mean blood pressure (MBP) and heart rate (HR).

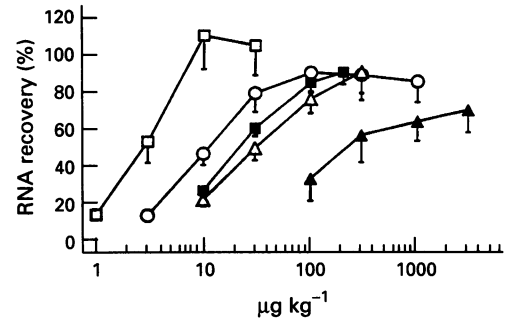

Figure 4 Dose-response curves ($n=5$) with increasing doses ($\mu g\,kg^{-1}$, i.v.) of (■) idazoxan, (□) methoxy-idazoxan, (○) atipamezole, (△) fluparoxan, (▲) BRL44408 illustrating the recovery of the renal nerve activity (RNA) after administration of clonidine ($10\,\mu g\,kg^{-1}$, i.v.) in presence of prazosin ($0.1\,mg\,kg^{-1}$, i.v.).

Table 2 Effect of idazoxan, methoxy-idazoxan (Meth-Idaz), BRL44408, atipamezole and fluparoxan on the bradycardia and sympatho-inhibition induced by clonidine (Clo) 10 $\mu g\ kg^{-1}$ in the rats treated with prazosin (0.1 mg kg^{-1}, i.v.)[a]

		Idazoxan	*Meth-Idaz*	*BRL44408*	*Atipamezole*	*Fluparoxan*
	Before (b.p.m.)	435 ± 25	394 ± 6	423 ± 13	416 ± 7	431 ± 12
	After Clo	-65 ± 14	-71 ± 6	-81 ± 9	-86 ± 12	-109 ± 16
HR	max.recovery (%)	76 ± 21[c]	123 ± 5 [c]	64 ± 11[c]	110 ± 13[c]	101 ± 5[c]
	IC_{50} $\mu g\ kg^{-1}$	28.5	4.29	10.13	15.3	44.9
	IC_{50} $\mu mol\ kg^{-1}$	0.14	0.018	0.047	0.072	0.187
	After Clo (%)	-73 ± 2	-75 ± 4	-71 ± 2	-84 ± 6	-84 ± 5
RNA	max.recovery (%)	90 ± 6[c]	110 ± 18[c]	71 ± 12[c]	90 ± 11[c]	91 ± 11[c]
	IC_{50} $\mu g\ kg^{-1}$	16.8	3	103.6	9.3	31
	IC_{50} $\mu mol\ kg^{-1}$	0.104	0.012	0.99	0.044	0.13

$n=5$

[a]The control value before clonidine (before), the effect of clonidine 10 min after its administration (after Clo), the maximal % of recovery, the IC_{50} values in $\mu g\ kg^{-1}$ and the IC_{50} values in $\mu mol\ kg^{-1}$ are indicated (the latter correspond to the doses inducing 50% of the maximal recovery). The control value of RNA before clonidine was taken as 100%. [b]The decrease in HR and RNA with clonidine was significant in all groups of rats ($P<0.05$). [c]The recovery is significant ($P<0.05$).

In five different groups of rats, these compounds were administered in the same dose-range as described above. After hexamethonium treatment the four imidazoline drugs and fluparoxan induced dose-dependent hypertensions (Figure 5a, Table 3). The maximal effect was reached 2 min after the injection; it remained stable with methoxy-idazoxan and fluparoxan but was reduced at 4 min with idazoxan, BRL44408 and atipamezole. Idazoxan did not significantly modify the heart rate (maximal effect: $+8\pm8$ b.p.m.) whereas methoxy-idazoxan, atipamezole and fluparoxan elicited a dose-dependent tachycardia (maximal effect respectively: $+72\pm7$, $+26\pm15$ and $+38\pm16$ b.p.m.); in contrast, BRL44408 induced a dose-related bradycardia (-27 ± 12 b.p.m. at the highest dose). To judge from EC_{50} values, the order of potency for the pressor response was methoxy-idazoxan > idazoxan > fluparoxan $\geq$ BRL44408 > atipamezole. Except for atipamezole, this order of potency corresponds to that of the antagonist effects of the compounds.

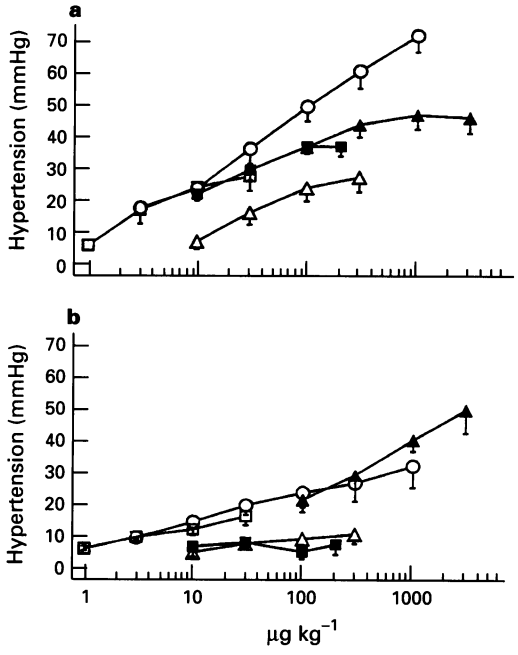

Figure 5 Dose-response curves ($n=5$) for (■) idazoxan, (□) methoxy-idazoxan, (○) atipamezole, (△) fluparoxan, (▲) BRL44408 on mean blood pressure (MBP), (a) after pretreatment with hexamethonium (10 mg kg^{-1}, i.v.) and (b) after pretreatment with hexamethonium and prazosin (0.1 mg kg^{-1}, i.v.).

Effect after pretreatment with hexamethonium and prazosin In order to confirm the involvement of vascular α_1-adrenoceptors in the hypertensive effect of idazoxan, methoxy-idazoxan, fluparoxan, BRL44408 and atipamezole, the experiments were repeated in five more groups of rats after treatment with both hexamethonium (10 mg kg^{-1}, i.v.) and prazosin (0.1 mg kg^{-1}, i.v.; 10 min after hexamethonium and 10 min before the administration of the antagonist tested). After hexamethonium and prazosin treatment, increasing doses of idazoxan, methoxy-idazoxan, atipamezole and fluparoxan elicited hypertensions that were significantly lower than those obtained in the animals treated only with hexamethonium (Table 3, Figure 5b). For idazoxan and methoxy-idazoxan, the maximal effects were lower and the EC_{50} values were higher in the presence of prazosin. For BRL44408, the EC_{50} value was higher but the same maximal response was reached. For fluparoxan and atipamezole, the maximal responses were decreased but comparable EC_{50} values were obtained. The tachycardia elicited by the compounds was not altered compared with hexamethonium treatment alone. Also the bradycardic effect of BRL44408 was not altered by the prazosin treatment.

Discussion

In the first part of our study we aimed to determine the relative potency of several α_2-adrenoceptor antagonists which differ in their structure and their affinity for α-adrenoceptor subtypes and imidazoline-sites in reversing the central action of clonidine.

The results indicate that in the normal rats, the drugs used do not equally reverse the inhibitory effect of clonidine on mean blood pressure, heart rate and sympathetic nerve activity. Yohimbine was not efficient at reversing the hypotension, but completely reversed the sympatho-inhibitory effect of clonidine. Pretreatment with yohimbine has been shown to block the hypotensive action of clonidine in hypertensive rats (Borkowski & Finch, 1979) and in the young rat (Gutkind *et al.*, 1986). However, yohimbine has also been shown to act as an antagonist at α_1-adrenoceptors (Drew, 1976; Shepperson *et al*, 1981; Doxey *et al.*, 1983) and it is possible that this effect causes a decrease in mean blood pressure thereby masking its α_2-adrenoceptor antagonist action.

Prazosin failed to alter any of the effects of clonidine and L-657,748 was the only compound which reversed both the hypotension, bradycardia and sympatho-inhibition induced by clonidine.

The four imidazoline drugs and fluparoxan all very potently reversed the hypotensive effect of clonidine but produced only a partial recovery of its sympatho-inhibitory action. After blockade of the α_1-adrenoceptors with prazosin, recovery of renal nerve activity was significantly better for all five drugs. These observations led us to hypothesize that the

Table 3 Effect of idazoxan, methoxy-idazoxan (Meth-Idaz), BRL44408, atipamezole and fluparoxan on mean blood pressure in rats ($n=5$) treated with hexamethonium 10 mg kg^{-1}, i.v. with or without prazosin (0.1 mg kg^{-1}, i.v.).

	After hexamethonium			*After hexamethonium and prazosin*			
	Maximal hypert. (mmHg)	EC_{50} (μg kg^{-1})	EC_{50} (μmol kg^{-1})	*Maximal hypert.* (mmHg)	EC_{50} (μg kg^{-1})	EC_{50} (μmol kg^{-1})	
Idazoxan	36±3	8.4	0.041	8±3	ND	ND	***
Meth-Idaz	27±5	2.32	0.0099	16±3	33.5	0.143	*
Fluparoxan	27±5	25.3	0.105	11±3	13.8	0.057	**
BRL44408	47±5	39.6	0.184	50±7	1062	4.93	NS
Atipamezole	71±5	89.9	0.42	32±7	37.5	0.18	***

$n=5$

*$P<0.05$, **$P<0.01$, ***$P<0.001$ for maximal hypertensive effect after hexamethonium and prazosin versus maximal hypertensive effect after hexamethonium

compounds probably have an action on peripheral α_1-adrenoceptors, in contrast to yohimbine; indeed, if they were to activate vascular α_1-adrenoceptors and merely induce hypertension, the baroreceptors would be activated leading to a decrease of the sympathetic nerve activity. This hypothesis was verified by the experiments on rats that were ganglion-blocked with hexamethonium. Under these conditions idazoxan, methoxy-idazoxan, atipamezole, BRL44408 and fluparoxan produced dose-related hypertensive effects and these pressor responses were reduced by prazosin treatment. These data then suggest that the greater recovery of mean blood pressure as compared to the recovery of sympathetic activity observed with these five drugs may be due to a peripheral hypertensive effect mediated through a direct activation of peripheral vascular α_1-adrenoceptors. Such an activation could be an indirect effect involving the blockade of presynaptic α_2-adrenoceptors and leading to an enhancement of neuronal release of noradrenaline. This explanation is unlikely because the hypertensive effect of the compounds was demonstrated in rats treated with hexamethonium and thus without functional sympathetic nerve terminals. Moreover a hypertensive effect of idazoxan through direct activation of α_1-adrenoceptors without involvement of presynaptic α_2-adrenoceptors has been described previously in the pithed rat (Dalrymple *et al.*, 1983; Paciorek & Shepperson, 1983).

The observation that idazoxan is not more effective in reversing the central action of clonidine than methoxy-idazoxan and that the four imidazoline structures are not more effective than L-657,743 and fluparoxan indicate that mainly α_2-adrenoceptors are involved in this action. These results are in agreement with the data of Head and collaborators (1993) obtained in the conscious rabbit who showed that idazoxan and methoxy-idazoxan equally reversed the action of intracisternal clonidine and also agree with the data of Hieble & Kolpak (1993) obtained in the anaesthetized hypertensive rat, indicating that idazoxan and SK&F86466 were equally effective in reversing the hypotensive action of systemic clonidine.

On the other hand, the present conclusions differ from those of Ernsberger *et al.* (1988, 1990); these authors compared the hypotensive effect of different drugs at the same dose and some of the differences in interpretation could be due to the potency rather than to the selectivity of the drugs. It is important to stress that in our present study, methoxy-idazoxan, which has an affinity for imidazoline-binding sites more than a 1000 times lower than that of idazoxan (Langin *et al.*, 1990; Mallard *et al.*, 1991; 1992) blocks the central effect of clonidine at doses 6 times lower than those of idazoxan, which is equipotent at imidazoline-binding sites and α_2-adrenoceptors (Wikberg *et al.*, 1991). A second point of the studies by Ernsberger *et al.*, was the correlation between the hypotensive effects of the drugs in the rat and their binding affinity at α_2-adrenoceptors and imidazoline sites in the bovine brain; later studies have illustrated that some of the drugs (e.g. oxymetazoline and clonidine) that decrease blood pressure very effectively in their studies have a good and specific affinity for α_{2A}-adrenoceptors in the rat (Harrison *et al.*, 1991; Uhlén *et al.*, 1992) and that clonidine binds more effectively to α_2-adrenoceptors than to imidazoline-binding sites (Langin *et al.*, 1990; Wikberg *et al.*, 1991). Binding data cannot easily be related to those obtained in functional studies, especially when species and tissue differences occur. It thus looks more reasonable to correlate some of our results with binding and autoradiographic data obtained in the rat brain; in the medulla of the rat, non-adrenoceptor binding sites appear to be located mainly in the area postrema (Mallard *et al.*, 1992), whereas α_2-adrenoceptors are located mainly in the nucleus tractus solitarii and the ventrolateral medulla (Rosin *et al*, 1993).

The α_2-adrenoceptors have been classified into four groups; however only α_{2A} and α_{2B}-adrenoceptors are currently clearly defined (see Bylund *et al*, 1995). The α_{2D}-adrenoceptors subtype seems to be the rat homologue of the human α_{2A}-adrenoceptor subtype and in most of the publications is also named α_{2A} in the rat. The α_{2C}-adrenoceptor remains linked to the α_{2B}-adrenoceptor subtype; it also has a good affinity for prazosin. Yohimbine binds with all α_2-adrenoceptor subtypes, perhaps with a better affinity for α_{2C}-adrenoceptors (Harrison *et al.*, 1991). No information was found about the affinities of fluparoxan for these subtypes. BRL44408 which has a good affinity for α_{2A}-adrenoceptors does not bind to α_{2B}-adrenoceptors (Young *et al.*, 1989). Prazosin has no affinity for the α_{2A}-adrenoceptor subtype (Bylund, 1985). Idazoxan, methoxy-idazoxan and clonidine display higher affinity for the α_{2A}-adrenoceptor subtype (Langin *et al.*, 1990; Harrison *et al.*, 1991; Uhlen & Wikberg, 1991). Consequently, the observations that prazosin administered at a high dose failed to alter the effects of clonidine and that the reversal elicited by idazoxan and methoxy-idazoxan were comparable may suggest that α_{2B}-adrenoceptors do not play a major role in the central effect of clonidine. These results would then favour the hypothesis that α_{2A}-adrenoceptors are involved in this mechanism. An alternative would be a role for α_{2C}-adrenoceptors. There are no pharmacological data concerning the α_{2C}-adrenoceptor subtype but methoxy-idazoxan, atipamezole, prazosin and yohimbine have been shown to bind to this subtype. The experiments of Uhlen *et al.* (1992) on α_{2A}-adrenoceptor and α_{2C}-adrenoceptor subtypes indicate a slightly higher affinity of clonidine and methoxy-idazoxan for α_{2A}-adrenoceptors, a higher affinity of BRL44408 for the α_{2A}-adrenoceptor subtype and a higher affinity of yohimbine and prazosin for α_{2C}-adrenoceptors. A higher affinity of clonidine for α_{2A}-adrenoceptors than for α_{2B} and α_{2C}-adrenoceptors was described by Harrison *et al.* (1991). At present there is no evidence to suggest a role for α_{2C}-adrenoceptors in the hypotensive action of clonidine.

The present results indicate that α_2-adrenoceptors are involved in the central sympatho-inhibitory and hypotensive effects of clonidine and indirectly suggest that this action may be mediated through stimulation of α_{2A}-adrenoceptors. These conclusions are in agreement with recent studies indicating that α_2-adrenoceptors are located in the ventrolateral medulla (Rosin *et al.*, 1993) and more precisely on bulbospinal presympathetic neurones in the rat (Guyenet *et al.*, 1994).

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