Desensitization and down-regulation of brain α_2 -adrenoceptors by centrally acting antihypertensive drugs

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Rabbits were treated with intravenous clonidine ($8 \mu\text{mol kg}^{-1} \text{ day}^{-1}$), guanabenz ($20 \mu\text{mol kg}^{-1} \text{ day}^{-1}$), rilmenidine ($80 \mu\text{mol kg}^{-1} \text{ day}^{-1}$) or vehicle via osmotic minipumps. After 6 days treatment mean arterial pressure (MAP), pressor responses to intravenous α -methyl noradrenaline and depressor responses to intracisternal clonidine were studied, and [^3H]-yohimbine binding to forebrain and hindbrain examined *in vitro*. Clonidine, guanabenz and rilmenidine had similar effects on MAP and caused a similar attenuation of the depressor response to intracisternal clonidine, but only guanabenz attenuated pressor responses to intravenous α -methyl noradrenaline. Rilmenidine had no effect on [^3H]-yohimbine binding to brain membranes. Clonidine treatment decreased binding in hindbrain while guanabenz treatment decreased binding in both fore- and hindbrain. Thus, the depressor effects of chronic treatment did not correlate with the effects on [^3H]-yohimbine binding sites in rabbit brain suggesting that the blood pressure lowering effects of many centrally acting antihypertensive drugs are not necessarily dependent on binding to the α_2 -adrenoceptor site labelled by [^3H]-yohimbine.

Keywords clonidine guanabenz rilmenidine α_2 -adrenoceptors

Introduction

Clonidine and other centrally acting antihypertensive drugs lower blood pressure via stimulation of α_2 -adrenoceptors in the brainstem (Kobinger, 1978). However, there are reports of differences between the central cardiovascular effects of α_2 -adrenoceptor agonists with phenylethylamine and imidazoline-like structures (Bousquet *et al.*, 1984) and some effects of the latter may be mediated via non-adrenergic receptors (Ernsberger *et al.*, 1987).

It has been suggested that a decrease in α_2 -adrenoceptor response and number may occur during chronic treatment with drugs such as clonidine which could contribute to the sympathetic hyperactivity observed following withdrawal (Finberg & Kopin, 1987). However, although changes in cardiovascular responses have been observed by several groups, changes in α_2 -adrenoceptor number have been harder

to show (Armstrong *et al.*, 1983) and may be less relevant than was previously supposed if some of the cardiovascular effects of clonidine-like drugs are mediated via non-adrenergic receptors.

We have compared treatment with three centrally acting antihypertensive drugs, clonidine, guanabenz and rilmenidine on cardiovascular responses and α_2 -adrenoceptor binding sites in rabbits.

Methods

In vitro IC_{50} values for displacement of the α_2 -adrenoceptor ligand [^3H]-yohimbine (5 nM) from homogenates of rabbit forebrain by clonidine, guanabenz and rilmenidine were calculated as previously described (Hamilton *et al.*, 1988).

In vivo groups of male New Zealand white rabbits (6–8) were treated with clonidine $8 \mu\text{mol kg}^{-1} \text{ day}^{-1}$, guanabenz $20 \mu\text{mol kg}^{-1} \text{ day}^{-1}$, rilmenidine $80 \mu\text{mol kg}^{-1} \text{ day}^{-1}$ or vehicle for 6 days. The drugs were administered into the femoral vein via osmotic minipumps (Alzet 2 mL l) as previously described (Yakubu *et al.*, 1988). The vehicle for clonidine and rilmenidine was sterile distilled water and for guanabenz distilled water/ethanol 3:2.

After 6 days treatment mean arterial pressure (MAP) was measured in conscious animals and pressor dose response curves to intravenous bolus doses of α -methyl noradrenaline (2.5–50 μg) constructed. The animals were then anaesthetised with sodium pentobarbitone and the depressor response to freehand injection of intracisternal clonidine examined. MAP was followed for 60–90 min before the animals were killed and forebrain and hindbrain removed. Membranes were prepared by homogenisation and centrifugation and stored at -70°C until used for radioligand binding studies with [^3H]-yohimbine (1.25–37 nM) (Yakubu *et al.*, 1988). Differences between vehicle and drug treated groups were examined using ANOVA or unpaired *t*-test as appropriate.

Results

In vitro radioligand and displacement studies

In vitro radioligand displacement studies in rabbit forebrain gave IC_{50} values for displacement of specific [^3H]-yohimbine binding of $5 \times 10^{-7} \text{ M}$, $1.5 \times 10^{-6} \text{ M}$ and $2 \times 10^{-6} \text{ M}$ for clonidine, guanabenz and rilmenidine respectively. Hill slopes were not significantly different from 1 for any of the compounds examined.

In vivo cardiovascular studies

MAP in the clonidine, guanabenz, rilmenidine, water vehicle and water/ethanol vehicle groups after 6 days treatment are shown in Table 1. Treatment resulted in falls in MAP -9 , -11 and -11 mm Hg for clonidine, guanabenz and rilmenidine relative to their respective control groups. The difference in MAP between drug treated and control groups was significant ($P < 0.05$) for rilmenidine (confidence intervals 17.6, 0.22) but not for guanabenz (confidence intervals 37.5, -1.3) or clonidine (16.4, -1.8).

Intracisternal clonidine injection caused a rapid fall in MAP in the vehicle treated groups which reached a maximum within 15 min and took 60–90 min to return to basal levels. Both

the extent and duration of the fall in blood pressure were attenuated in the drug treated animals. The maximum fall in MAP in each group is shown in Table 1.

Intravenous bolus doses of α -methyl noradrenaline (2.5–50 μg) caused a dose-related increase in mean arterial pressure in all animals. However, the response to any given dose was significantly less in the animals treated chronically with guanabenz. In this group the pressor dose-response curve was shifted to the right with a dose ratio of 3.0 relative to the control curve.

α_2 -adrenoceptor binding site number

Specific [^3H]-yohimbine binding to both forebrain and hindbrain was decreased in the guanabenz treated group compared with its control group (Table 1). The maximum number of binding sites in hindbrain was also reduced in the clonidine treated group but rilmenidine had no significant effect on binding to either forebrain or hindbrain. No drug treatment had any significant effect on K_D .

Discussion

The doses for *in vivo* treatment were selected on the basis of their *in vitro* affinity for the [^3H]-yohimbine binding site. Using this regime clonidine, guanabenz and rilmenidine had similar effects on MAP and the depressor response to intracisternal clonidine. However, only guanabenz attenuated the pressor response to α -methyl noradrenaline. This may relate to the drugs' differing affinities for peripheral vascular α_2 -adrenoceptors. Pressor responses to intravenous guanabenz are preferentially blocked by α_2 -adrenoceptor selective antagonists such as yohimbine and idazoxan while pressor responses to intravenous clonidine and rilmenidine are more easily blocked by the α_1 -adrenoceptor antagonist, prazosin (Hannah *et al.*, 1984; Xiao & Rand, 1989).

Although the effects of chronic treatment with clonidine, guanabenz and rilmenidine on centrally mediated depressor responses were similar, marked differences in their effects on [^3H]-yohimbine binding to brain membranes were observed with rilmenidine having no effect on binding in either brain region, clonidine treatment resulting in a 37% decrease in binding in hindbrain and guanabenz 55% and 44% decreases in fore- and hindbrain respectively. Guanabenz probably has a longer plasma half-life than either clonidine or rilmenidine and differences in metabolism and distribution of the

Table 1 Effect of 6 days treatment with clonidine, guanabenz, rilmenidine or vehicle on cardiovascular responses and [^3H]-yohimbine binding

Group	Basal MAP (mm Hg)	Decrease in MAP relative to vehicle (mm Hg)	Maximum fall in MAP after intracisternal clonidine	Pressor response to 10 μg αMeNa	B_{max} (fmol mg^{-1} protein) Forebrain	Hindbrain
Vehicle water	72 $\pm$ 10	—	27 $\pm$ 6	39 $\pm$ 5	88 $\pm$ 22	83 $\pm$ 23
Clonidine (8 $\mu\text{mol kg}^{-1} \text{ day}^{-1}$)	63 $\pm$ 8	-9	12 $\pm$ 3**	38 $\pm$ 4	98 $\pm$ 42	52 $\pm$ 11*
Rilmenidine (80 $\mu\text{mol kg}^{-1} \text{ day}^{-1}$)	61 $\pm$ 6*	-11	10 $\pm$ 5**	36 $\pm$ 5	90 $\pm$ 46	74 $\pm$ 16
Vehicle water/ethanol	81 $\pm$ 9	—	26 $\pm$ 10	37 $\pm$ 10	118 $\pm$ 22	108 $\pm$ 24
Guanabenz (20 $\mu\text{mol kg}^{-1} \text{ day}^{-1}$)	70 $\pm$ 10	-11	14 $\pm$ 8*	26 $\pm$ 4*	53 $\pm$ 13**	60 $\pm$ 27*

Significantly decreased compared with appropriate control group.

* $P < 0.05$; ** $P < 0.01$; $\alpha\text{MeNa} = \alpha\text{-methyl noradrenaline}$

drugs *in vivo* may contribute to differences in down-regulation. However the differences in down-regulation were not reflected in differences in the depressor effects observed on chronic treatment and these studies are consistent with the hypothesis that the blood pressure lowering effects of many centrally acting anti-

hypertensive drugs are not solely dependent on binding to the α_2 -adrenoceptor site labelled by [^3H]-yohimbine.

This work was supported in part by Institut de Recherches Servier France. M.A.Y. is supported by the University of Maiduguri, Nigeria.

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