Br. J. Pharmac. (1987), **91**, 481–486

A study of the action of clonidine on secretion from the adrenal medulla in dogs

F. Anglade, *L. Dang Tran, G. De Saint Blanquat, *G. Gaillard, *C. Michel-Damase, *J.L. Montastruc, *P. Montastruc, *M. Rostin & *[1]M.A. Tran

INSERM U 87 Institut de Physiologie, Université Paul Sabatier, Rue François Magendie and *Laboratoire de Pharmacologie Médicale et Clinique, UA 644 du CNRS, Faculté de Médecine, 37, Allées Jules-Guesde, 31073 Toulouse Cédex France

1 The effects of clonidine on adrenal catecholamine (adrenaline and noradrenaline) secretion were investigated in chloralose-anaesthetized dogs.

2 Intravenous administration of clonidine (10 and 20 $\mu g\,kg^{-1}$) induced a decrease in both adrenal catecholamine secretion rates and cardiovascular parameters (blood pressure and heart rate). In contrast, a dose of 5 $\mu g\,kg^{-1}$ was ineffective.

3 Intracisternal clonidine (in a lower dose of 3 $\mu g\,kg^{-1}$) also decreased adrenaline and noradrenaline release from the adrenal gland.

4 Clonidine failed to modify adrenal catecholamine release evoked by electrical stimulation of the splanchnic nerve.

5 These results demonstrate that clonidine decreases adrenaline release from the adrenal gland through a central and not a peripheral mechanism in dogs. This action might contribute to its antihypertensive effects.

Introduction

Clonidine, a centrally antihypertensive agent and a selective α_2-adrenoceptor agonist, decreases blood pressure via a reduction in sympathetic tone (Schmitt, 1977). However, as well as its action on noradrenaline, some studies have suggested an effect on adrenaline secretion. In normotensive rats Shimamura *et al.* (1981) found a decreased secretion of both adrenaline and noradrenaline into the adrenal vein; this effect was also observed in spontaneously hypertensive rats (Togashi, 1983). Indirect evidence was also found in man; the drug was reported to reduce the excretion of urinary adrenaline (Hökfelt *et al.*, 1970). These actions of clonidine agree with the known effects of α-adrenoceptor agonists: for example Gutman & Boonyaviroj (1974) showed that noradrenaline suppressed adrenaline secretion induced by insulin hypoglycaemia in rats.

Moreover, previous experiments from our group have shown that in adrenal demedullated dogs with virtually no circulating adrenaline, clonidine induced both a delayed hypotensive effect and a significant tachycardia when compared with normal control dogs (Montastruc *et al.*, 1985). On the other hand, Brown & Macquin (1981) have suggested that adrenaline has an important role in the pathogenis of essential hypertension. Thus, in the light of this hypothesis, it appears to be important to differentiate between antihypertensive drugs that are able to act on adrenal secretion rate, which led us to study, in dogs, the effect of clonidine on the function of the adrenal medulla.

[1] Author for correspondence

Methods

Animals and general procedure

Mongrel dogs (12 to 25 kg) of either sex were anaesthetized with α-chloralose (80 $mg\,kg^{-1}$ i.v.), curarized by gallamine (2 $mg\,kg^{-1}$ i.v.) and artificially ventilated with an Ideal Palmer pump (insuflated air volume: 15 $ml\,kg^{-1}$ with a frequency of 16 per min). Fully adequate anaesthesia was maintained by an injection of 15–20 mg of chloralose each hour. Carotid arterial blood pressure and heart rate were respectively measured with a Beckman pressure transducer and a tachocardiometer triggered by electrocardiogram

(lead II) linked to a chart recorder. Body temperature of the animals was maintained at a constant level around 38.5 C and arterial pH was monitored using a Metrohm pH meter.

Adrenal venous blood sampling

After median laparotomy, the right adrenal vein was dissected and its collaterals occluded. The animals were heparinized (500 u kg^{-1} every 2 h) and the adrenal vein was occluded at the level of its junction with the inferior vena cava, whereas the other end was cannulated in order to enable blood sampling. When not being sampled, adrenal venous blood was directly returned to the femoral vein via a cannula. Blood sampling started 25 min after adrenal vein cannulation. Each blood sample (5 ml) was collected in a tube containing anticoagulant heparin (0.1%) and immediately frozen. Plasma was immediately separated by centrifugation and stored below −80°C. Plasma catecholamine (adrenaline and noradrenaline) concentrations were measured by high pressure liquid chromatography with electrochemical detection (coulometry). Adrenal plasma flow rate (ml kg^{-1} min^{-1}) was measured and catecholamine outputs (adrenaline and noradrenaline) expressed as ng kg^{-1} min^{-1}.

Drug administration

Clonidine (Boehringer) was injected either peripherally (5, 10 and 20 μg kg^{-1}) through the saphenous vein, or intracisternally (1 and 3 μg kg^{-1}) through a spinal needle using a constant volume (0.4 ml). In the control experiments the animals received only saline as a sham injection.

Section of the splanchnic nerve

About 1 h before the beginning of blood collection, the right great splanchnic nerve was dissected and incised below the diaphragm. The peripheral end of the right splanchnic nerve was stimulated via a bipolar stainless steel electrode. The stimuli consisted of 5 min trains of rectangular pulses, supramaximal intensity, duration 0.1 ms, frequency 5 Hz. Clonidine or saline were administered by the intravenous route 8 min before the electrical stimulation. Adrenal venous blood was collected 10 min before stimulation and 2 min after the beginning of stimulation. Thus blood samples were taken 10 min after clonidine, i.e. when the effect of clonidine was maximal.

Statistical analysis

Results are presented as mean values ± s.e.mean. Significance was estimated by use of Student's *t* test for unpaired comparisons or the Wilcoxon test. When the *P* value was greater than 0.05 the difference was not considered to be significant.

Results

Control experiments

In six control dogs, catecholamine secretion rates were evaluated just before (0), 10 and 20 min after saline injection in order to see if these values changed during the whole experiment. Similar measurements were made of adrenal flow rate. These times (10 and 20 min) were chosen as a result of preliminary experiments from our laboratory investigating the time course of the hypotensive and bradycardic effect of clonidine under similar experimental conditions (Montastruc *et al.*, 1985). Figure 1 shows that both the catecholamine (adrenaline and noradrenaline) secretion rate and adrenal plasma flow rate remained constant. Before saline injection (time 0), mean outputs of adrenaline and noradrenaline were respectively 38.8 ± 12.8 and 4.9 ± 1.5 ng kg^{-1} min^{-1}. Thus, in anaesthetized dogs, adrenaline secretion from the adrenal medulla is about 8 times higher than noradrenaline secretion.

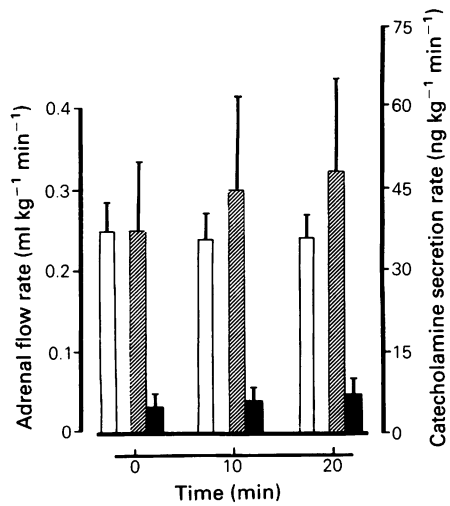

Figure 1 Adrenal catecholamine levels and adrenal venous plasma flow rate in control (sham) experiments. Adrenal plasma flow rate (ml kg^{-1} min^{-1}; open columns), adrenaline release (ng kg^{-1} min^{-1}; hatched columns) and noradrenaline release (ng kg^{-1} min^{-1}; solid columns) were expressed as mean values with vertical lines indicating s.e.mean. Time 0 was just before saline injection. Measured values at time 10 and 20 min after saline injection were compared with the control values (time = 0) by use of the Wilcoxon test; $n = 6$.

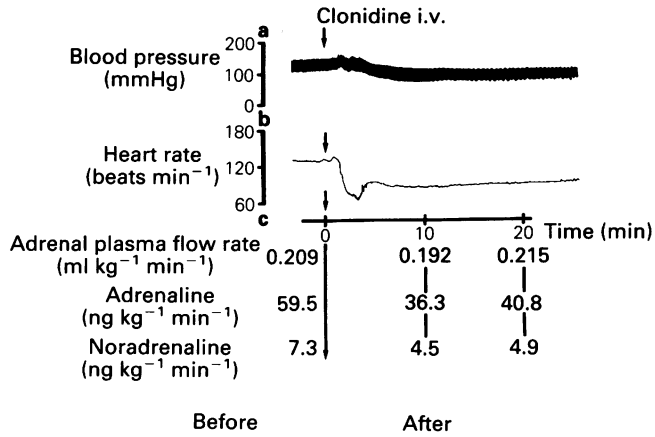

Figure 2 Typical record of the effect of clonidine (10 μg kg^{-1} i.v.) on blood pressure (mmHg) (a), heart rate (beats min^{-1}) (b), adrenal plasma flow rate and adrenal catecholamine release (c). The values were obtained before, 10 and 20 min after clonidine injection.

Effect of intravenous clonidine

These experiments were performed in 3 groups of 5 (for 5 μg kg^{-1}) or 6 (for 10 and 20 μg kg^{-1}) dogs. Mean resting catecholamine levels were 49.8 ± 13.3 and 6.2 ± 1.5 ng kg^{-1} min^{-1} ($n = 17$) for adrenaline and noradrenaline respectively in this series of experiments. Adrenal plasma flow rate was 0.241 ± 0.017 ml kg^{-1} min^{-1}. Clonidine (5, 10, 20 μg kg^{-1} i.v.) induced a dose-dependent decrease in the catecholamine secretion rate 10 and 20 min after drug administration. The maximal decrease was observed 10 min after clonidine injection, as shown in Figure 2 (typical record after 10 μg kg^{-1} i.v. clonidine). Simultaneously, adrenal plasma flow rate decreased after 10 and 20 μg kg^{-1} of clonidine, this effect being due to the postsynaptic α_2-adrenoceptor vasoconstrictor properties of clonidine (Schmitt, 1977). This last action was maximal 10 min after clonidine, as indicated in Figure 2.

Figure 3 shows the decreased flow rate of catecholamines and adrenal plasma 10 min after clonidine administration. We note that this decrease affected both adrenaline and noradrenaline to a similar extent. Moreover, when peripheral plasma catecholamine levels were simultaneously measured ($n = 5$), clonidine (10 μg kg^{-1} i.v.) induced a decrease in noradrenaline as well as adrenaline plasma levels: resting values were respectively 358.4 ± 88.9 and 263.5 ± 65.7 pg ml^{-1}; i.e. the drug induced a 62.1 ± 8.4 and 61.6 ± 8.1% decrease for noradrenaline and adrenaline, respectively.

Resting systolic and diastolic blood pressures were 156.1 ± 4.1 and 112.4 ± 4.3 mmHg respectively ($n = 17$). Heart rate was 170.7 ± 6.9 beats min^{-1} before drug administration. Intravenous clonidine elicited a short lasting increase in blood pressure, the maximal effect being observed 2 to 3 min after injection, as shown in Figure 2 after 10 μg kg^{-1}. This hypertensive phase could persist until 5 to 6 min after 20 μg kg^{-1}. Secondly, clonidine induced a decrease in

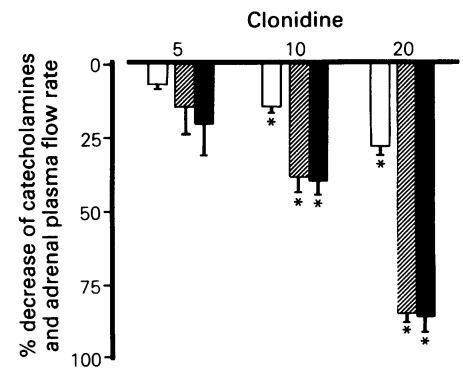

Figure 3 Effects of several doses of clonidine (5, 10 and 20 μg kg^{-1} i.v.) on catecholamine release from the adrenal gland and adrenal plasma flow rate measured 10 min after clonidine. Adrenal plasma flow rate: open columns; adrenaline release: hatched columns; noradrenaline release: solid columns. Catecholamine secretion and adrenal flow measured 10 min after clonidine were expressed as percentage decrease from each control value obtained before clonidine injection. Each column represents the mean values of 5 to 6 experiments for each of the 3 doses and vertical lines show s.e.mean *$P < 0.05$ compared with control values by use of the Wilcoxon test.

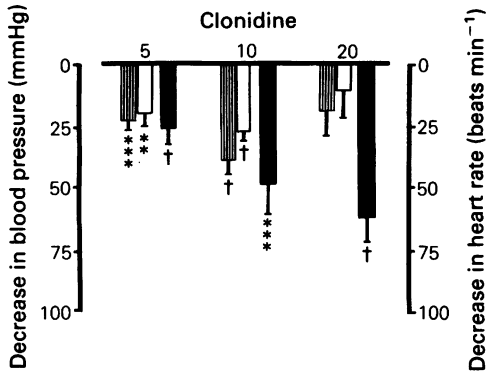

Figure 4 Effects of several doses of clonidine (5, 10 and 20 $\mu g\,kg^{-1}$ i.v.) on systolic (vertically hatched columns) and diastolic (open columns) blood pressure and heart rate (solid columns). Blood pressure and heart rate were measured before and 10 min after clonidine. The columns represent the mean decrease in blood pressure and in heart rate obtained from 5 to 6 experiments for each of the 3 doses and vertical lines show s.e.mean. The values obtained 10 min after clonidine injection were compared with control values by use of Student's t test for paired comparisons. $^{**}P<0.02$, $^{***}P<0.01$; $\dagger P<0.001$.

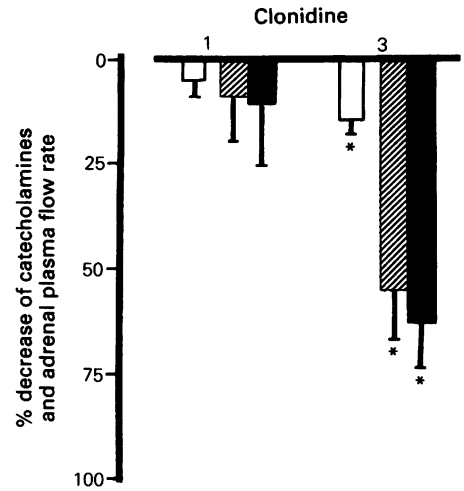

Figure 5 Effects of intracisternal administration of clonidine (1 and 3 $\mu g\,kg^{-1}$) on adrenal catecholamine release and adrenal plasma flow rate (measured 10 min after clonidine). Adrenal plasma flow rate (open columns); adrenaline release (hatched columns) and noradrenaline release (solid columns). The values, evaluated 10 min after clonidine, were expressed as % decrease from the control values. Each column represents the mean values of 4 (for 1 $\mu g\,kg^{-1}$) or 6 (for 3 $\mu g\,kg^{-1}$) experiments. Vertical lines show s.e.mean $^{*}P<0.05$ compared with control values by use of the Wilcoxon test.

blood pressure, observed under our experimental conditions for at least 20 min (see Figure 2). Simultaneously, heart rate decreased. Figure 4 shows the effects of intravenous clonidine (5 to 20 $\mu g\,kg^{-1}$) on the systolic and diastolic blood pressures and heart rate measured 10 min after drug administration. Only clonidine induced changes in heart rate were dose-dependent. However, the decrease in blood pressure elicited by the higher dose of clonidine (20 $\mu g\,kg^{-1}$ i.v.) was not significant. This last effect could be explained by the postsynaptic α_2-agonist property of clonidine which counteracted its sympatholytic actions.

Effect of intracisternal clonidine

In order to investigate the participation of a central mechanism in this clonidine-induced decrease in adrenaline secretion, the effects of low doses (1 and 3 $\mu g\,kg^{-1}$) of clonidine (inactive when injected by peripheral route: see Figures 3 and 4) administered by the intracisternal route were studied. In preliminary experiments, we found that intracisternal saline administration did not change the cardiovascular or biochemical parameters. Figures 5 and 6 show that only 3 $\mu g\,kg^{-1}$ of clonidine induced a decrease in both catecholamine release from the adrenal medulla and cardiovascular parameters, whereas 1 $\mu g\,kg^{-1}$ was inactive. We were unable to explain the slight but

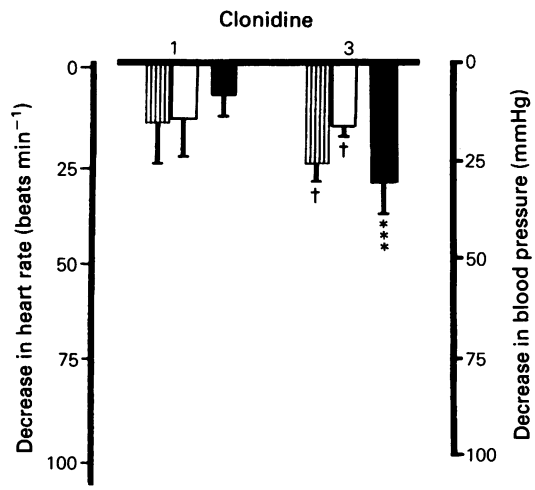

Figure 6 Effects of intracisternal injection of clonidine (1 and 3 $\mu g\,kg^{-1}$) on systolic (vertically hatched columns) and diastolic (open columns) blood pressure, and heart rate (solid columns) (10 min after drug administration). As in Figure 4 the columns represent the mean values of 5 to 6 experiments and vertical lines show s.e.mean. $^{***}P<0.01$; $\dagger P<0.001$, compared to control values (Student's t test for paired data).

significant decrease in adrenal plasma flow rate observed after intracisternal injection of 3 $\mu g\,kg^{-1}$ clonidine.

Effect of clonidine on catecholamine secretion evoked by electrical stimulation of the splanchnic nerve

This part of the investigation was performed on 18 dogs. When the great splanchnic nerve was sectioned, resting (before saline or clonidine administration) adrenal outputs were 3.5 ± 0.6 and 1.8 ± 0.3 ng $kg^{-1}\,min^{-1}$ ($n = 18$) for adrenaline, respectively. Thus, adrenal denervation significantly reduced the adrenaline secretion rate by ten to fifteen fold. Splanchnic nerve stimulation significantly increased adrenal catecholamine release. For example, 2 min after the beginning of nerve stimulation, adrenal secretion rates were 188.3 ± 23.4 and 50.1 ± 5.6 ng $kg^{-1}\,min^{-1}$ for adrenaline and noradrenaline respectively in control experiments (see Table 1). Simultaneously, electrical stimulation induced a slight but significant increase in adrenal plasma flow rate ($20.5 \pm 7.3\%$ when compared with control values). Table 1 clearly shows that clonidine pretreatment failed to modify adrenaline and noradrenaline release.

Discussion

As far as we know, the effects of clonidine on adrenaline release from the adrenal gland have never been studied in anaesthetized and curarized dogs. Under our experimental conditions (full anaesthesia and curarization with gallamine), catecholamine levels remained constant in control animals during the whole experiment. Arterial pH was between 7.40 and 7.42. Gallamine prevents the variations in carotid arterial pressure induced by respiratory irregularities without inducing ganglioplegy and Sumikawa *et al.* (1979) claim it does not alter the secretion rate of adrenaline from the gland.

Our present data clearly demonstrate that clonidine reduces adrenaline as well as noradrenaline release from the adrenal medulla in dogs. This result agrees with the data of Shimamura *et al.* (1981) in normotensive rats and those of Togashi (1983) in spontaneously hypertensive rats. Thus, clonidine seems to exert a similar effect on the function of the adrenal medulla in both species (rat and dog). However, we suggest that the mechanism of this action differs between the two species: in our experiments in dogs, the effect of clonidine is due to a central mechanism, since low doses of clonidine (3 $\mu g\,kg^{-1}$), ineffective by the peripheral route, were active after intracisternal administration. The present data exclude a peripheral component since, after section of the splanchnic nerve, the drug failed to reduce catecholamine release elicited by splanchnic nerve stimulation. In contrast, in hypertensive rats, Togashi (1983) suggested, besides a central site of action, the involvement of a peripheral mechanism in the action of clonidine on the function of the adrenal medulla.

Clonidine induced adrenal vasoconstriction as well as a decrease in catecholamine release. However, it is important to note that this clonidine-induced fall in adrenal plasma flow rate (for example 11.4% for 10 $\mu g\,kg^{-1}$) is insufficient to explain the inhibitory effect of clonidine on adrenal secretion, since the mean % decrease in adrenaline was $41.2 \pm 3.1\%$ after this dose.

Similar to results obtained by Edwards (1982) in the conscious calf, we also observed, under our experimental conditions, a significant increase in adrenal venous flow rate during splanchnic nerve stimulation. According to Edwards, the decreased adrenal vascular resistance could be explained by vasoactive intestinal polypeptide release from the splanchnic nerve.

Table 1 Effects of clonidine on catecholamine secretion evoked by splanchnic nerve stimulation.

	Control group		*Clonidine groups*			
			10 $\mu g\,kg^{-1}$		20 $\mu g\,kg^{-1}$	
	Before stimulation	*During stimulation*	*Before stimulation*	*During stimulation*	*Before stimulation*	*During stimulation*
Adrenaline	3.2 ± 0.7	188.3 ± 23.4	3.4 ± 0.7	161.2 ± 30.1	3.9 ± 0.8	197.6 ± 34.3
Noradrenaline	1.5 ± 0.3	50.1 ± 5.6	1.9 ± 0.5	42.6 ± 8.3	2.1 ± 0.5	45.2 ± 8.1

The control group received only saline ($n = 7$). Two other groups of animals were treated with clonidine (10 $\mu g\,kg^{-1}$ i.v., $n = 5$ and 20 $\mu g\,kg^{-1}$ i.v.; $n = 6$). Catecholamine levels were measured 10 min before nerve stimulation (before stimulation) and 2 min after the beginning of stimulation (during stimulation). Each value represents the mean ± s.e.mean secretion rate in ng $kg^{-1}\,min^{-1}$. Catecholamine release values during splanchnic nerve stimulation and after clonidine (10 or 20 $\mu g\,kg^{-1}$) were compared with values obtained during nerve stimulation in the control group by use of Student's unpaired t test. Note that in clonidine and control groups, basal (before simulation) values of catecholamine release were not different.

The present work questions the presence of α_2-adrenoceptors modulating adrenaline release on adrenal chromaffin cells, although it is accepted that prejunctional α_2-adrenoceptors are present on sympathetic nerve terminals where they modulate neurotransmitter release (Starke, 1977; Langer, 1980). Several authors have investigated this problem using different species and *in situ* or *in vitro* experimental models: chromaffin cells in culture, isolated adrenal glands. In rabbits, Collett *et al.* (1981, 1982) did not find any evidence for the existence of α_2-adrenoceptors modulating adrenaline release. These results were confirmed by Nakazato *et al.* (1984) using cat perfused adrenal glands. In bovine adrenal glands, several conflicting results have been obtained: Greenberg & Zinder (1982) and Wada *et al.* (1982) suggested the involvement of an α-adrenoceptor-mediated mechanism for the fine regulation of secretion from the adrenal medulla. In contrast, Powis & Baker (1986) failed to demonstrate such a mechanism. Finally, the work of Togashi (1983) suggested that the clonidine-induced decrease was mediated by activation of α_2-adrenoceptors located on adrenal cells. Our work did not find evidence of such adrenal α_2-adrenoceptors in dogs as previously described in cats and rabbits.

In conclusion, our study demonstrates that clonidine decreases adrenaline release from the adrenal gland through a central (and not a peripheral) mechanism in dogs. From a pharmacological point of view, the present study and the data presented above suggest that clonidine decreases sympathetic tone not only by an action on the neuro-neuronal sympathetic pathways (through a reduction in noradrenaline release from the sympathetic nerve endings; Schmitt, 1977) but also on the neuro-adrenal sympathetic axis (through a decrease in function of the adrenal medulla). This action on the adrenal medulla might contribute significantly to its antihypertensive effect. Thus, clonidine appears to be a suitable drug to investigate the hypothesis of the involvement of adrenaline in the pathophysiology of high blood pressure (Brown & Macquin, 1981).

The authors gratefully acknowledge the help of Prof. Charlet (Dpt of Statistics, CHU Toulouse) and Mr Duplantier and Mrs Gayraud for technical help. This work was partly supported by Boehringer (France).

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(Received July 30, 1986. Revised November 24, 1986. Accepted March 5, 1987.)