

INFLUENCE OF ANALGESIC ANTIPYRETICS ON THE CENTRAL CARDIO-VASCULAR EFFECTS OF CLONIDINE IN RATS¹

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ABSTRACT

The centrally acting antihypertensive drug clonidine has been found to stimulate the synthesis of PGF_{2α} in the brain. Centrally administered PGF_{2α}, in turn, induces rises of blood pressure and heart rate. We therefore studied the influence of inhibitors of prostaglandin (PG) synthesis on the cardiovascular effects of clonidine in urethane-anaesthetised rats. Pretreatment with indomethacin or paracetamol (100 µg/rat into the fourth cerebral ventricle) antagonised the central hypotensive effect of clonidine (0.125-16.0 µg/rat into the fourth cerebral ventricle). The bradycardic effect of centrally administered clonidine was, however, enhanced by pretreatment with paracetamol but not influenced by indomethacin pretreatment. Sodium meclofenamate (100 µg/rat into the fourth cerebral ventricle) did not significantly affect the clonidine-induced changes in blood pressure and heart rate.

These results suggest that the clonidine-induced hypotension on one hand and bradycardia on the other hand may be mediated by partly different mechanisms. An interference of the formation of PGF_{2α} with the cardiovascular effects of clonidine cannot be completely excluded since paracetamol pretreatment potentiated the bradycardic effect of clonidine. However, inhibitors of PG synthesis did not enhance but antagonised the hypotensive effect of clonidine. Therefore it is likely that the synthesis of PGF_{2α} does not interfere with the hypotensive effect of clonidine. Moreover, the antagonism of the hypotensive effect by inhibitors of PG synthesis suggests that some hypotensive metabolite of arachidonic acid in the brain could be involved in the central hypotensive effect of clonidine.

INTRODUCTION

Clonidine is a centrally acting antihypertensive drug (2,3) which stimulates the synthesis of prostaglandin F_{2α} (PGF_{2α}) in the brain (4) and in various other mammalian tissues (4,5,6). Intracerebroventricularly administered PGF_{2α} induces strong dose-related rises of blood pressure and heart rate in rats (7). Therefore it seemed possible that the clonidine-induced synthesis of prostaglandins might influence the cardiovascular effects of this agent.

We now report the influence of some inhibitors of PG synthesis on the centrally induced cardiovascular effects of clonidine in anaesthetised rats.

¹A preliminary of this work was presented at the 7th International Congress of Pharmacology in Paris (Sirén, A.-L. and H. Karppanen 1978).

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MATERIALS AND METHODS

Female Sprague-Dawley rats (200-280 g) were used. The rats were accommodated to standard ambient conditions for at least one week before the experiments. The lights were on from 6 a.m. to 6 p.m. and the room was completely dark during the remaining 12 hours. The temperature was kept at 22 °C and the relative humidity at 40%. The rats received standard rat pellets (Hankkija Oy, Helsinki) and tap water *ad libitum*. The rats were anaesthetised with urethane (1.5 g/kg i.p.). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously. The mean arterial blood pressure was measured from the left femoral artery by means of a pressure transducer (Harvard apparatus 377). The heart rate was calculated from the pulse waves. The left femoral vein was cannulated for intravenous injections. The rats were mounted in a stereotaxic instrument and tilted caudally so that the body formed an angle of 10 degrees with the horizontal plane. An injection needle was introduced into the fourth ventricle of the brain. A polyethylene catheter, filled with the drug or control solution to be infused, was attached to the needle. The desired amount of the solution to be infused was allowed to flow slowly by the virtue of hydrostatic pressure. The infusion was stopped by closing the upper end of the catheter. The proper position of the needle tip was ascertained at the end of each experiment by an injection of dye (methylene blue) into the fourth cerebral ventricle. The body temperature was measured rectally with a temperature recorder (ELLAB instruments, type Z8, Copenhagen), a probe being introduced 5 cm into the rectum. A 60 W heating lamp was placed 15 cm above the rat. This distance of the heating lamp was adequate to keep the body temperature of the control rats at $36.1 \pm 0.4^{\circ}\text{C}$ (mean \pm s.e.m.) in an ambient temperature of 22 °C which was kept constant automatically by air conditioning.

Administration of drugs

Clonidine hydrochloride (Boehringer Ingelheim) was dissolved in 0.9% (w/v) NaCl (saline). Since the region of the fourth cerebral ventricle is the most likely site of the cardiovascular actions of clonidine (8) the drugs were administered into the fourth ventricle of the brain. Increasing doses of clonidine were injected at 20 minutes intervals in order to obtain a cumulative dose-response curve. This interval was chosen because it has been previously shown that the maximum hypotensive effect of clonidine is reached in 20 minutes (9). Indomethacin (Orion Pharmaceutical Co., Helsinki) was dissolved in 0.025 N NaOH and paracetamol (Medipolar-Farmos Co., Turku) in distilled water. Sodium meclofenamate monohydrate (Parke, Davis & Co.) was dissolved in saline. The influence of indomethacin, paracetamol and sodium meclofenamate on the central actions of clonidine was studied after the administration of these drugs into the fourth ventricle of the brain at a dose of 100 µg/rat 20 minutes before the start of the administration of the first dose of clonidine. The injections of the drugs into the fourth cerebral ventricle were given in a volume of 10 µl each. In each case the control animals received the same volume of the appropriate solvent at the same pH as the drug solution. Simultaneously with each central injection the animals received 0.1 ml of saline intravenously.

The Student's t-test was used to calculate the statistical significance of the differences between the control and experimental groups.

RESULTS

I Effects of the inhibitors of prostaglandin synthesis on blood pressure, heart rate and body temperature

After the central administration neither indomethacin nor sodium meclofenamate had any significant effects of their own on the baseline values for blood pressure, heart rate or body temperature. Paracetamol, however, induced a rise of heart rate (Table I).

Table I. Baseline values for blood pressure (B.P.), heart rate (H.R.) and body temperature (B.T.) in indomethacin, paracetamol and sodium meclofenamate pretreated rats and their controls immediately before the administration of clonidine into the fourth cerebral ventricle. The values represent means \pm SD means. Each group comprised 6 rats. The difference in the baseline value for heart rate between paracetamol pretreated and control group is significant at the $p < 0.05$ level.

Pretreatment	B.P. (mm Hg)	H.R. (beats/min)	B.T. (°C)
Vehicle	100 \pm 10	370 \pm 50	36.1 \pm 0.2
Indomethacin (100 μ g/rat)	90 \pm 5	380 \pm 40	36.4 \pm 0.2
Vehicle	100 \pm 10	350 \pm 50	36.0 \pm 0.7
Paracetamol (100 μ g/rat)	95 \pm 10	430 \pm 70	36.5 \pm 0.5
Vehicle	100 \pm 15	320 \pm 40	36.0 \pm 0.9
Sodium meclofenamate (100 μ g/rat)	100 \pm 10	360 \pm 50	36.8 \pm 0.5

II Influence of the inhibitors of prostaglandin synthesis on the central cardiovascular effects of clonidine

A. Effect of indomethacin

Central pretreatment with indomethacin, 100 μ g/rat, antagonised the hypotensive effect of clonidine shifting the dose-response curve for clonidine to the right (Fig. 1). Centrally administered indomethacin did not significantly affect the bradycardic effect of clonidine (Fig. 2).

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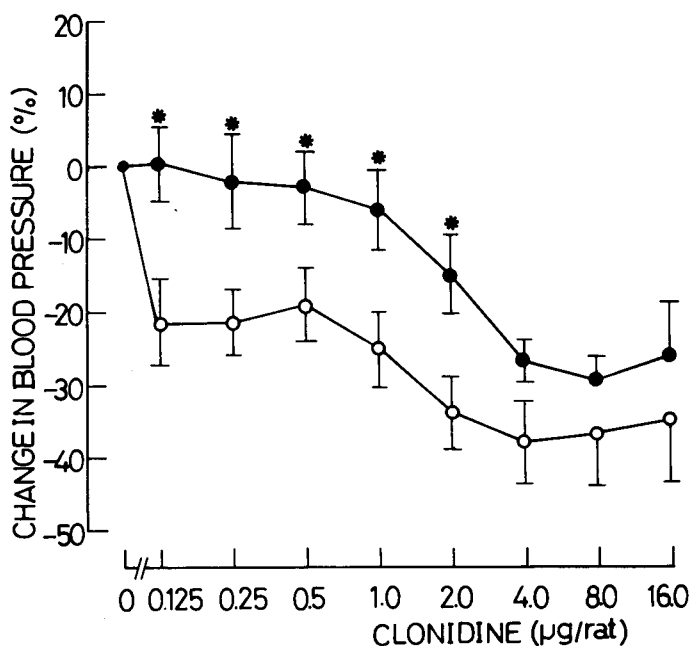


Figure 1. Influence of central pretreatment with indomethacin on the hypotensive effect of clonidine. Indomethacin, 100 µg/rat, (●—●) or vehicle (○—○) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the indomethacin pretreated and control groups is significant at the $p < 0.05$ level at the doses of 0.125-2.0 µg/rat of clonidine. Vertical bars indicate s.e.m. Each group comprised 6 rats.

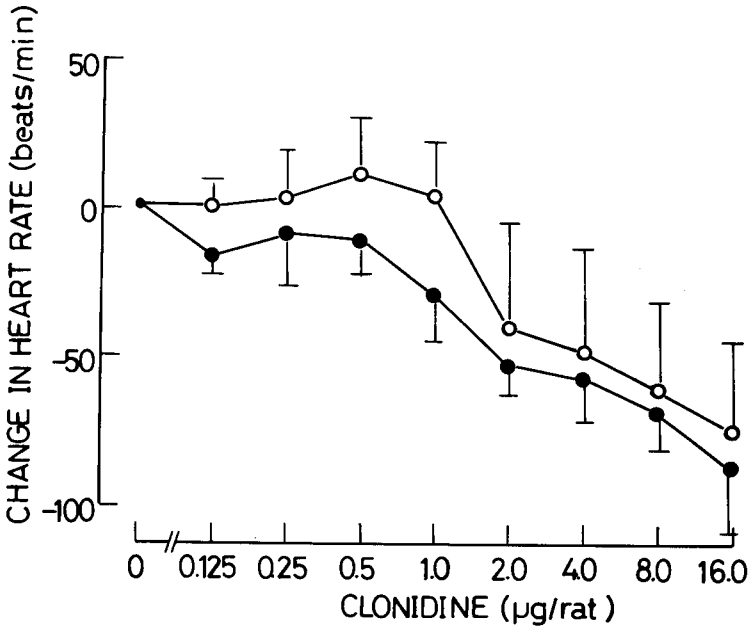


Figure 2. Influence of central pretreatment with indomethacin on the bradycardic effect of clonidine. Indomethacin, 100 µg/rat (●—●), or vehicle (○—○) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the indomethacin pretreated and control groups is not statistically significant. Vertical bars indicate s.e.m. Both groups comprised 6 rats.

B. Effect of paracetamol

Central pretreatment with paracetamol, 100 µg/rat, antagonised the hypotensive effect of clonidine (Fig. 3) but enhanced the clonidine-induced bradycardia (Fig. 4).

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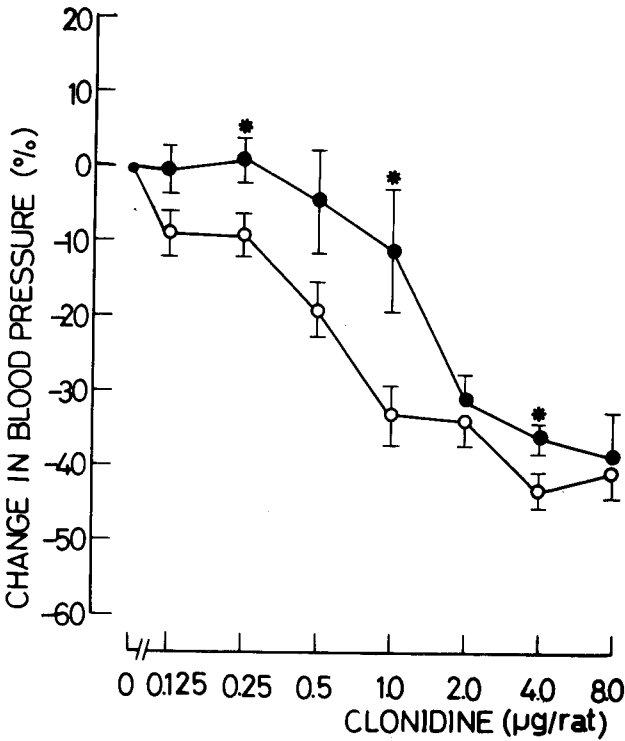


Figure 3. Influence of central pretreatment with paracetamol on the hypotensive effect of clonidine. Paracetamol, 100 µg/rat, (●—●) or vehicle (○—○) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the paracetamol pretreated and control groups is significant at the $p < 0.05$ level at the doses of 0.25, 1.0 and 4.0 µg/rat of clonidine. Vertical bars indicate s.e.m. Both group comprised 6 rats.

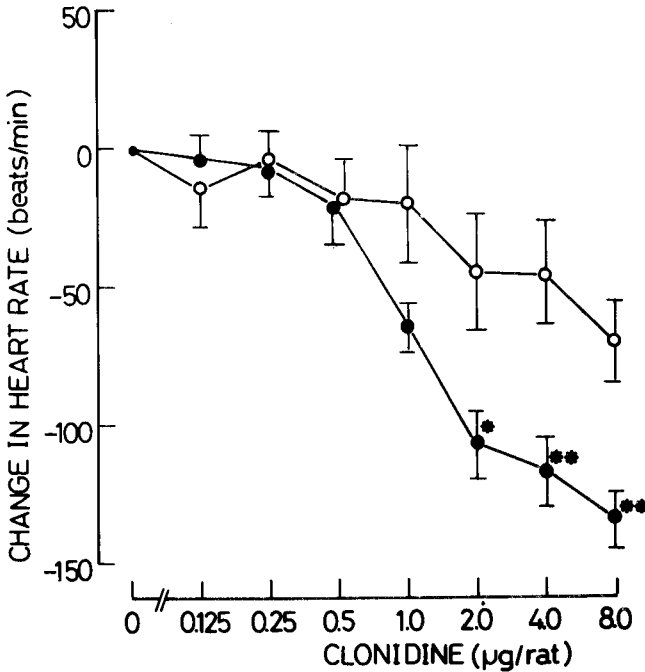


Figure 4. Influence of central pretreatment with paracetamol on the bradycardic effect of clonidine. Paracetamol, 100 µg/rat, (●—●) or vehicle (○—○) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the paracetamol pretreated and control groups is significant at the $p < 0.05-0.01$ level at the doses of 2.0-8.0 µg/rat of clonidine. Vertical bars indicate s.e.m. Both groups comprised 6 rats.

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C. Effect of sodium meclofenamate

Central pretreatment with sodium meclofenamate, 100 $\mu\text{g}/\text{rat}$, slightly potentiated the hypotensive effect of clonidine (Fig. 5) but did not affect the bradycardic effect of clonidine (Fig. 6).

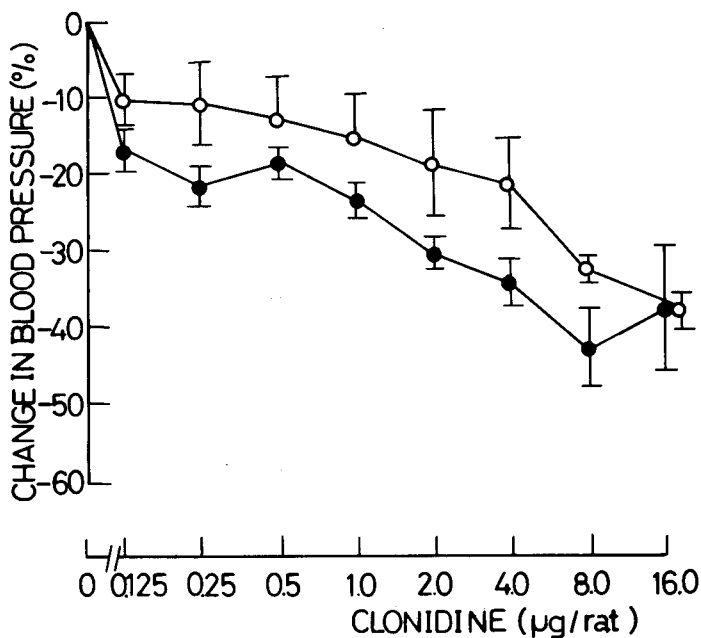


Figure 5. Influence of central pretreatment with sodium meclofenamate on the hypotensive effect of clonidine. Sodium meclofenamate, 100 $\mu\text{g}/\text{rat}$, (●—●) or vehicle (○—○) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the sodium meclofenamate pretreated and control groups is not statistically significant. Vertical bars indicate s.e.m. Both groups comprised 6 rats.

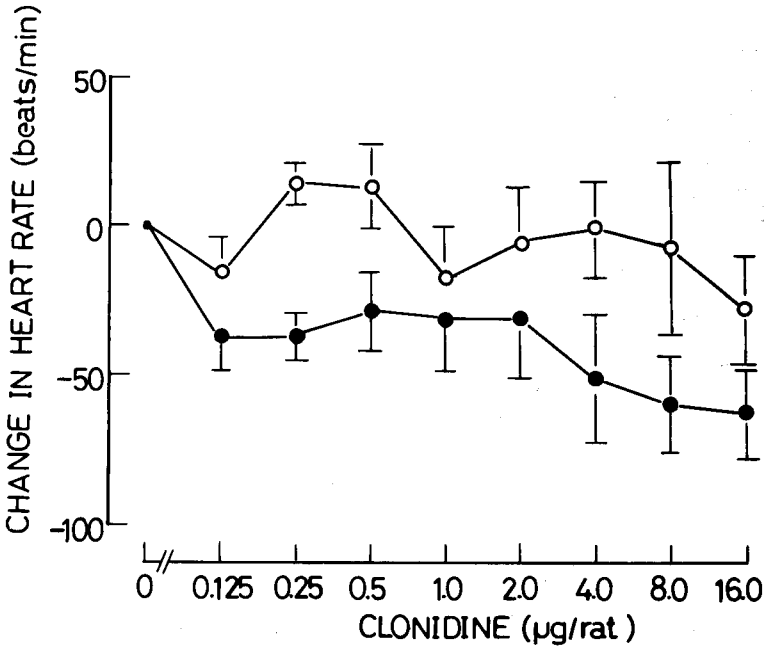


Figure 6. Influence of central pretreatment with sodium meclofenamate on the bradycardic effect of clonidine. Sodium meclofenamate, 100 µg/rat (●—●), or vehicle (o—o) was administered into the fourth cerebral ventricle 20 minutes before commencement of the administration of clonidine into the fourth cerebral ventricle. The doses are indicated on the abscissa. The difference between the sodium meclofenamate pretreated and control groups is not statistically significant. Vertical bars indicate s.e.m. Both groups comprised 6 rats.

DISCUSSION

Intracerebroventricular pretreatment of the rats with indomethacin or paracetamol antagonised the centrally induced hypotensive effect of clonidine. However, the bradycardic effect of clonidine tended to be enhanced by indomethacin and was clearly potentiated by paracetamol. Thus the central mechanisms involved in the hypotensive effect of clonidine on one hand and in its bradycardic effect on the other hand, appear to be partly different. This suggestion is also supported by the finding that the histamine H₂-receptor antagonist metiamide inhibits the hypotensive but not the bradycardic effect of clonidine (10). In agreement with our results it has been shown that in conscious rabbits the pretreatment with indomethacin or acetylsalicylic acid inhibits the hypotensive effect of various intravenously administered antihypertensive drugs, including clonidine (11). Indomethacin, acetylsalicylic acid and paracetamol share the ability to inhibit the synthesis of prostaglandins

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in the brain (12, 13). These results would therefore suggest that the synthesis of prostaglandins in the brain is associated with the hypotensive effect of clonidine. This assumption is, however, contradicted by the finding that pretreatment with sodium meclofenamate, another inhibitor of arachidonic acid metabolism (12), did not influence the central cardiovascular effects of clonidine in the same way as indomethacin and paracetamol did.

It has been reported that clonidine stimulates the synthesis of $\text{PGF}_{2\alpha}$ in rat and rabbit brain *in vivo* (4, 5). However, centrally administered $\text{PGF}_{2\alpha}$ exerts hypertensive and tachycardic effects (7). The administration of $\text{PGF}_{2\alpha}$ intracerebroventricularly prevented the hypotensive effect of clonidine and had a tachycardic effect also in the presence of clonidine (Table II). Inhibitors of prostaglandin biosynthesis should enhance rather than antagonise the hypotensive effect of clonidine if an increased formation of $\text{PGF}_{2\alpha}$ was involved in this effect of clonidine. Unlike the hypotensive effect, the bradycardic effect of clonidine was, in fact, enhanced by paracetamol pretreatment and tended to be enhanced also by indomethacin and sodium meclofenamate pretreatment. Therefore the present findings on blood pressure cannot, while those on heart rate could be explained by clonidine-induced formation of $\text{PGF}_{2\alpha}$ in the brain.

Table II Clonidine-induced changes in blood pressure and heart rate in $\text{PGF}_{2\alpha}$ treated and control rats. Clonidine and saline or clonidine and $\text{PGF}_{2\alpha}$ were administered intracerebroventricularly (i.c.v.). The values represent means \pm SD means 20 minutes after the administration of the drugs. Each group comprised 6 rats. The differences between the two groups are significant at the $p < 0.001$ level.

Group	Change in	
	blood pressure (mm Hg)	heart rate (beats/min)
Clonidine (0.125 $\mu\text{g}/\text{rat}$ i.c.v.) +saline i.c.v.	-16 \pm 5	-20 \pm 40
Clonidine (0.125 $\mu\text{g}/\text{rat}$ i.c.v.) + $\text{PGF}_{2\alpha}$ (1.0 $\mu\text{g}/\text{rat}$ i.c.v.)	0 \pm 5	90 \pm 20

Clonidine also stimulates the synthesis of PGE in rat brain *in vitro* and in rabbit heart *in vivo* (4, 5). It also increases the excretion of PGE from dog kidney (6), and prostaglandins have been implicated in the renal vascular effects of clonidine (14). However, PGE_2 i.c.v. induces similar rise of blood pressure as $\text{PGF}_{2\alpha}$ does (15). Thus the present blood pressure findings can hardly be attributed to the formation of PGE by clonidine in the brain either.

Recently it was shown that prostacyclin i.c.v. induces hypotension in conscious rats (16), and we have recently observed the same effect also in urethane-anaesthetised rats (unpublished results). Prostacyclin is also known to be formed in the brain (17). Indomethacin and paracetamol inhibit the prostaglandin cyclo-oxygenase in the brain and thus the synthe-

sis of both prostacyclin and several other metabolites of arachidonic acid (12, 13, 18). Therefore the possibility exists that the formation of prostacyclin or some other product of arachidonic acid, other than $\text{PGF}_2\alpha$ or PGE_2 , in the brain might contribute to the hypotensive effect of clonidine.

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