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Evidence for a central α-sympathomimetic action of clonidine in the rat

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The antihypertensive effects of clonidine (0·15 mg kg^{-1}, i.p.) were studied in conscious DOCA/saline hypertensive rats having chronically implanted arterial cannulae. The response to clonidine was markedly reduced by simultaneously administered desipramine (3 mg kg^{-1}, i.p.), antagonized dose-dependently by piperoxan (2–10 mg kg^{-1}, i.v.) and prevented by pretreatment with phentolamine (0·2 mg, i.c.v.). Pretreatment with 6-hydroxydopamine (3 × 250 μg, i.c.v.), haloperidol (1 mg kg^{-1}, i.p.), *p*-chloro-*N*-methylamphetamine (3·5 mg kg^{-1}, i.p.) or 5, 6-dihydroxytryptamine (50 μg and 25 μg, i.c.v.) did not significantly modify the antihypertensive response. It is concluded that the antihypertensive response to clonidine is mediated via stimulation of central α-adrenoceptors and is independent of central dopaminergic receptors and intact central serotoninergic neurons. The necessity for intact central noradrenergic neurons remains uncertain.

Clonidine is a potent antihypertensive agent which acts on the bulbar sympathetic centres leading to a reduction in peripheral sympathetic tone (Kobinger & Walland, 1967). This effect is thought to be due to a central α-sympathomimetic action since piperoxan and yohimbine, which cross the blood brain barrier, and intraventricularly injected phentolamine antagonize the hypotensive effect of clonidine (Schmitt, Schmitt & Fénard, 1971; Haeusler & Finch, 1972; Haeusler, 1973). The hypotensive response is also antagonized by pretreatment with desipramine (Reid, Briant & Dollery, 1973; Van Spanning & Van Zwieten, 1973) or 6-hydroxydopamine (6-OH-DA) (Dollery & Reid, 1973) which would suggest a presynaptic site of action of clonidine (Starke & Altmann, 1973). The central regulation of blood pressure may also involve dopaminergic (Bolme & Fuxe, 1971) and serotoninergic pathways (Ito & Schanberg, 1972; Florez & Armijo, 1974; Neumayr, Hare & Franz, 1974). Indeed 5-hydroxytryptamine (5-HT) levels in the pons-medulla region are elevated by clonidine (Maj, Baran & others, 1973).

The present studies were designed to investigate the rôle of central dopaminergic receptors, and serotoninergic and noradrenergic neurons in the mediation of the antihypertensive response to clonidine in DOCA/saline hypertensive rats. A preliminary report of this work was presented as a Communication to the British Pharmaceutical Conference, London (September 1973) (Bucher, Buckingham & others, 1973).

MATERIALS AND METHODS

Experimental hypertension was induced in male Sprague-Dawley rats, 120–150 g, by unilateral nephrectomy under ether anaesthesia and subcutaneous implantation of two compressed tablets each containing 25 mg deoxycorticosterone acetate (DOCA). The drinking water was replaced by 0·9% w/v saline. Four to six weeks later, a polythene cannula was implanted in the abdominal aorta under ether anaesthesia for

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the direct recording of blood pressure using a modification of the technique of Weeks & Jones (1960). In some cases, a cannula was also inserted into the abdominal vena cava for the intravenous administration of drugs. Intracerebroventricular (i.c.v.) injections were made via a cannula implanted in the lateral brain ventricle according to Hayden, Johnson & Maickel (1966). The rats were allowed 1–2 days to recover before use.

Blood pressure was recorded in conscious, unrestrained hypertensive animals using a Statham P 23 Db. pressure transducer connected to a Grass Model 7 B Polygraph and heart rate was counted from the record after increasing the chart speed. The animals were housed individually and the blood pressure was recorded at 2 and 1 h before dosing. Only animals with a mean arterial pressure greater than 145 mm Hg were selected at this stage. The blood pressure was recorded again immediately before dosing, and all subsequent blood pressure differences were related to this value. The mean blood pressure was calculated for each treatment group and the significance of differences was evaluated by Student's *t*-test.

The drugs used were deoxycorticosterone acetate (Organon); desmethylimipramine hydrochloride (CIBA—Geigy U.K.); phentolamine methanesulphonate (CIBA); haloperidol (Janssen); clonidine hydrochloride (Boehringer); piperoxan, *p*-chloro-*N*-methylamphetamine, 5,6-dihydroxytryptamine creatinine sulphate, 6-hydroxydopamine hydrobromide (F. Hoffmann-La Roche, Basle). 5,6-Dihydroxytryptamine and 6-hydroxydopamine were dissolved in 0·1 mg ml^{-1} ascorbic acid and nitrogen-bubbled 0·01 N hydrochloric acid respectively and their doses are expressed as base weight. Other drugs were dissolved in 0·9% w/v saline and their doses are expressed as mg of the salt per kg body weight (mg kg^{-1}). Animals which received 5,6-dihydroxytryptamine (5,6-HT) showed marked reductions in food and fluid intake and became aggressive in response to external stimuli. These together with control animals were given glucose daily (12 ml kg^{-1}, i.p. of a 5% w/v solution in water) for the duration of pretreatment.

RESULTS

In conscious DOCA/saline hypertensive rats, clonidine (0·05–0·2 mg kg^{-1}, i.p.) produced a dose-dependent fall in blood pressure (20–80 mm Hg) which was often accompanied by a bradycardia (40–200 beats min^{-1}). An initial rise in blood pressure of 10–20 mm Hg was observed in some animals for a period of 10–60 min. The effect of various pretreatments were studied using a fixed dose of clonidine (0·15 mg kg^{-1}, i.p.) which gave reproducible antihypertensive responses (Fig. 1–4) without any undue stress to the animals. Piperoxan (2, 5 or 10 mg kg^{-1}, i.v.) 1 h previously produced a dose-dependent antagonism of the response to clonidine, the highest dose completely preventing the fall in blood pressure at 1 h and causing a marked, but incomplete, reduction in the response up to 4 h after clonidine (Fig. 1A).

Piperoxan elicited a slight antihypertensive response (10–28 mm Hg) throughout the experiment. Phentolamine (0·2 mg, i.c.v.) 1 h before clonidine produced total blockade of the response for 3 h (Fig. 1B). Phentolamine, when given alone (0·2 mg, i.c.v.) produced an antihypertensive response (20–34 mm Hg) and a bradycardia (30–70 beats min^{-1}) which persisted for the duration of the experiment; marked behavioural changes manifested by aggression and increased sensitivity to tactile and auditory stimuli were also observed in these rats. Desipramine (3 mg kg^{-1}, i.p.) administered simultaneously with clonidine prevented the antihypertensive response (Fig. 2A), but

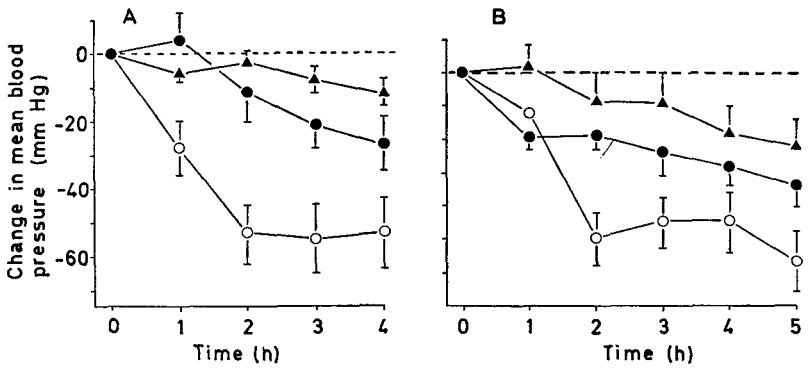

FIG. 1.A. Antagonism of the antihypertensive response to clonidine in conscious DOCA/saline rats by pretreatment with piperoxan. Clonidine, 0·15 mg kg^{-1}, i.p. 60 min after saline (0·9%w/v) 1 ml kg^{-1}, i.v. (n = 9), ○——○; saline (0·9% w/v), 2 ml kg^{-1}, i.p. 60 min after piperoxan, 10 mg kg^{-1}, i.v. (n = 9) ▲——▲; clonidine, 0·15 mg kg^{-1}, i.p. 60 min after piperoxan, 10 mg kg^{-1} i.v. (n = 8), ●——●. In this and subsequent figures each point is a mean with vertical bars indicating the standard error.

B. Prevention of the antihypertensive response to clonidine by pretreatment with phentolamine. Clonidine 0·15 mg kg^{-1} i.p. 60 min after saline (0·9% w/v) 20 μl i.c.v. (n = 10) ○——○; saline (0·9% w/v) 2 ml kg^{-1} i.p. 60 min after phentolamine 0·2 mg, i.c.v. (n = 9) ●——●; clonidine 0·15 mg kg^{-1} i.p. 60 min after phentolamine 0·2 mg, i.c.v. (n = 11) ▲——▲.

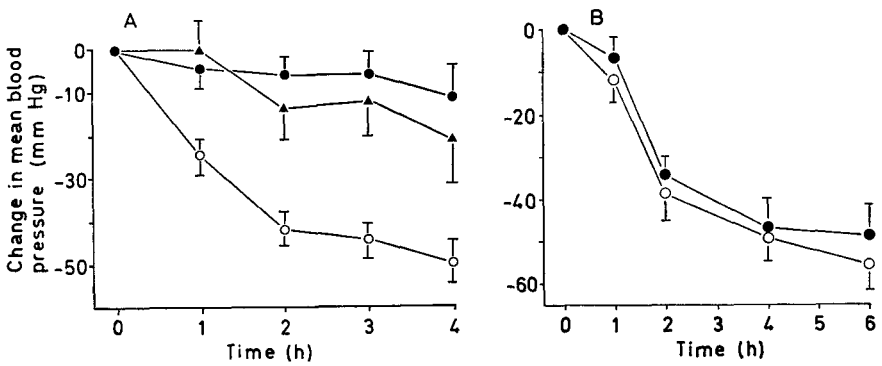

FIG. 2.A. Antagonism of the antihypertensive response to clonidine in conscious DOCA/saline hypertensive rats by desipramine. Clonidine, 0·15 mg kg^{-1}, i.p., and saline (0·9% w/v) 2 ml kg^{-1}, i.p., administered simultaneously (n = 23) ○——○; desipramine, 3 mg kg^{-1}, i.p. and saline (0·9% w/v) 2 ml kg^{-1}, i.p. administered simultaneously (n = 19) ●——●; clonidine 0·15 mg kg^{-1}, i.p. administered simultaneously with desipramine, 3 mg kg^{-1} i.p. (n = 20), ▲——▲.

B. The antihypertensive response to clonidine after pretreatment with 6-OH-DA. Pretreatment with 6-OH-DA, 250 μg, i.c.v., 2, 4 and 6 days before clonidine, 0·15 kg^{-1} i.p. (n = 14), ●——●; pretreatment with vehicle, 20 μl i.c.v., 2, 4 and 6 days before clonidine, 0·15 mg kg^{-1} i.p. (n = 14), ○——○.

pretreatment with 6-OHDA (250 μg, i.c.v. 2, 4 and 6 days previously) was without effect (Fig. 2B). The blood pressure responses of individual animals which received desipramine (3 mg kg^{-1}, i.p.) showed marked variation. This was also apparent in the animals that were dosed with both desipramine and clonidine.

Haloperidol (1 mg kg^{-1}, i.p.), 1 h before the injection of clonidine, produced a small but not significant, reduction in the response (Fig. 3). Haloperidol alone reduced blood pressure slightly (10–20 mm Hg) for the duration of the experiment and induced behavioural changes such as catatonia and decreased motor activity.

The involvement of central serotoninergic neurons was investigated by pretreating rats with 5,6-HT (50 μg and 25 μg, i.c.v., 4 and 2 days before clonidine respectively). The antihypertensive response to clonidine was not significantly modified by this pretreatment (Fig. 4A).

Pretreatment with *p*-chloro-*N*-methylamphetamine (3·5 mg kg^{-1} i.p. 3 days previously) also had no significant effect on the response to clonidine (Fig. 4B).

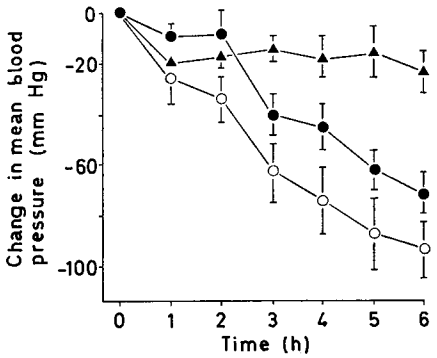

FIG. 3. The effect of haloperidol on the antihypertensive response to clonidine. Clonidine, 0·15 mg kg^{-1}, i.p., 60 min after saline (0·9% w/v), 2 ml kg^{-1}, i.p. (n = 10), ○——○; saline (0·9% w/v), 2 ml kg^{-1}, i.p. 60 min. after haloperidol, 1 mg kg^{-1}, i.p. (n = 8), ▲——▲; clonidine, 0·15 mg kg^{-1}, i.p. 60 min after haloperidol, 1 mg kg^{-1}, i.p. (n = 8), ●——●.

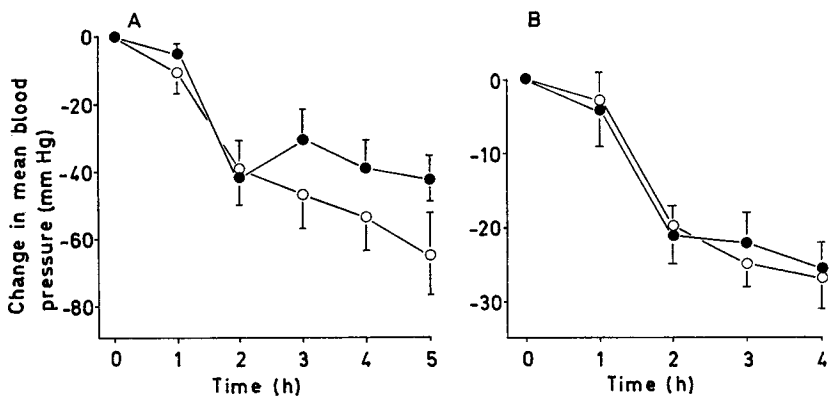

FIG. 4.A. The antihypertensive response to clonidine after pretreatment with 5,6-HT. Pretreatment with 5,6-HT, 25 μg, i.c.v., 2 days and 50 μg, i.c.v., 4 days before clonidine, 0·15 mg kg^{-1}, i.p. (n = 11), ●——●; pretreatment with vehicle 20 μl i.c.v. 2 and 4 days before clonidine, 0·15 mg kg^{-1}, i.p. (n = 11), ○——○.

B. The effect of *p*-chloro-*N*-methylamphetamine pretreatment on the antihypertensive response to clonidine. Clonidine, 0·15 mg kg^{-1}, i.p. (n = 30), ○——○; clonidine, 0·15 mg kg^{-1}, i.p., 3 days after *p*-chloro-*N*-methylamphetamine, 3·5 mg kg^{-1}, i.p.(n = 25), ●——●.

DISCUSSION

In conscious DOCA/saline hypertensive rats, clonidine produced a marked and sustained fall in blood pressure often accompanied by a bradycardia. Similar findings were observed in anaesthetized and conscious rats, cats and rabbits (Hughes, 1968; Schmitt, Schmitt & Fénard, 1971; Dollery & Reid, 1973; Haeusler, 1973; Poyser, Shorter & Whiting, 1974; Finch, 1974).

The antihypertensive effect of clonidine was abolished by phentolamine (i.c.v.) and

a dose-dependent reduction of the response was produced by intravenous piperoxan. Since piperoxan readily passes the blood brain barrier, the results support the hypothesis for a central α-adrenoceptor stimulating action of clonidine (Schmitt & others, 1971) and are consistent with the results of previous studies using piperoxan and yohimbine in anaesthetized cats (Schmitt, Schmitt & Fénard, 1973). The hypotension and bradycardia produced by phentolamine (i.c.v.) has also been observed after intracisternal administration to anaesthetized rats (Ito & Schanberg, 1974) and may be due to a partial agonist effect (Schmitt & others, 1973). In a preliminary study Bucher & others (1973) reported desipramine (1–3 mg kg^{-1}, i.p.) to have no effect on the antihypertensive response to clonidine in DOCA/saline hypertensive rats. These experiments have been repeated using larger numbers of animals and the results demonstrate a highly significant reduction in the response to clonidine by desipramine (3 mg kg^{-1}, i.p.). The possibility that this dose of tricyclic antidepressant was sufficient to block α-adrenoceptors was investigated in 6 anaesthetized DOCA/saline hypertensive rats. Blood pressure responses to intravenous injections of noradrenaline (0·5–2 μg, kg^{-1}) were potentiated by intravenously injected desipramine (3 mg kg^{-1}). In other experiments, the antihypertensive response to pargyline (100 mg kg^{-1}, i.p.) in DOCA/saline rats was not reduced by desipramine (3 mg kg^{-1}, i.p.) administered simultaneously, thereby eliminating the possibility of central α-adrenoceptor blocking activity (unpublished observations).

The discrepancy between the present findings with desipramine and our earlier results may be due to the smaller group sizes used previously and also to the extreme variability in the blood pressure responses of individual rats to desipramine. The present findings confirm those in conscious rabbits (Reid & others, 1973) and anaesthetized cats (Van Spanning & Van Zwieten, 1973) where the hypotensive responses to clonidine were abolished by pretreatment with desipramine. The results contrast with those obtained in anaesthetized rabbits (Hoefke & Warnke-Sachs, 1974), conscious cats (Finch, 1974) and a previous study in DOCA/saline hypertensive rats (Scholtysik & Saltzmann, 1973) in which clonidine responses were unaffected by desipramine. Biochemical evidence shows that the doses of desipramine used in all these studies were below the dose (25 mg kg^{-1}, i.p.) reported to block central adrenergic uptake mechanisms (Carlsson, Fuxe & others, 1966).

In our experiments, the antihypertensive response to clonidine was not modified by 6-OH-DA (i.c.v.) pretreatment which is known to produce widespread destruction of central noradrenergic neurons (Uretsky & Iversen, 1970; Haeusler, Finch & Thoenen, 1972). A similar pretreatment regimen was shown to abolish the antihypertensive response to α-methyldopa in genetically hypertensive rats (Finch & Haeusler, 1973) and markedly reduce the antihypertensive response to pargyline in DOCA/saline rats (unpublished observation). The present results in rats are in agreement with those of Haeusler & Finch (1972) and in rabbits by Warnke & Hoefke (unpublished) but do not confirm studies by Dollery & Reid (1973) in conscious and anaesthetized rabbits. Our results with desipramine and 6-OH-DA do not lend total support to either an exclusively pre-synpatic or post-synaptic site of action for clonidine in central noradrenergic neurons. To postulate a pre-synaptic action it would be necessary to suggest an action for desipramine on noradrenergic neurons which are insensitive to 6-OH-DA in the rat whilst support for a post-synaptic action of clonidine would depend upon an unknown action of desipramine. We have no satisfactory explanation of this inconsistency.

The antihypertensive response to clonidine was not significantly modified by pretreatment with haloperidol, a central dopaminergic receptor antagonist. Although dopaminergic pathways may be important in the central regulation of blood pressure (Bolme & Fuxe, 1971) the results suggest such pathways are not involved in mediating the antihypertensive response to clonidine in the rat.

The response to clonidine was unaffected by pretreatment with 5,6-HT, an agent known to produce a widespread destruction of serotoninergic neurons (Baumgarten, Evetts & others, 1972) or *p*-chloro-*N*-methylamphetamine, indicating that an interaction with serotoninergic neurons is unlikely to be an important mediating pathway. Both treatments are known to produce marked depletions of brain 5-HT and although the pons/medulla region is least affected, even so the uptake of 5-HT in this region is markedly reduced (Baumgarten & others, 1972; Miller, Cox & others, 1970; Björkland, Nobin & Stenevi, 1973). The present results do not, however, rule out an interaction with central 5-HT receptors.

It is concluded that the antihypertensive action of clonidine in DOCA/saline hypertensive rats is mediated via central α-adrenoceptors and is independent of dopaminergic receptors and intact serotoninergic neurons. The necessity for intact noradrenergic neurons remains uncertain.

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