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EFFECT OF CLONIDINE ON THE 5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLEACETIC ACID BRAIN LEVELS*

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Abstract—The effects of clonidine on the brain levels of 5-HT and 5-HIAA in rats and mice were studied. Clonidine did not change the levels of 5-HT and 5-HIAA in the whole brains of either animal species but the 5-HT concentrations were elevated in rat brain pons + medulla oblongata. Clonidine antagonized the increase in the brain 5-HIAA levels induced by apomorphine in rats and mice. The decrease in the 5-HT level and the increase in the 5-HIAA level observed in rats after L-dopa (given with peripheral decarboxylase inhibitor RO 4-4602) were counteracted by clonidine.

CLONIDINE is regarded as an agent which directly activates the central NA recepters.[1–4] Combined treatment with apomorphine and clonidine results in a greater stimulation of the locomotor activity than the administration of apomorphine alone.[1,5,6] In a previous study[6] we have shown that clonidine given alone does not produce a motor stimulation. It inhibits the locomotor activity of normal mice, and does not affect that depressed by reserpine, CA synthesis inhibitors or agents blocking the central CA receptors. The same effect in reserpinized animals was described by other authors.[1,5]

It is not clear why the activation of NA receptors by clonidine in apomorphine-treated animals results in locomotor stimulation whereas in untreated animals it inhibits or does not change locomotor activity. This suggests that besides the NA mechanism clonidine may affect other receptor or receptors.

In this study we examined the effects of clonidine on the brain levels of 5-HT and 5-HIAA in normal animals, and those treated with apomorphine or with L-dopa together with an inhibitor of peripheral decarboxylase, RO 4-4602.[7]

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats, weighing 120–160 g, and on male Albino–Swiss mice, weighing 18–24 g. The brain levels of 5-HT and 5-HIAA were determined spectrofluorimetrically according to the methods described by Maickel *et al.*[8] and Miller *et al.*[9] respectively. The animals were killed by cervical dislocation. In some experiments rat brains were dissected into separate regions using the method of Popov *et al.*[10] The excised brains were placed on solid carbon dioxide and dissected

Abbreviations used: CA, catecholamines; L-dopa, β-(3,4-dihydroxyphenyl)-L-α-alanine; 5-HIAA, 5-hydroxy-3-indoleacetic acid; 5-HT, 5-hydroxytryptamine; MAO, monoaminooxydase; NA, noradrenaline; RO 4-4602, *N*-(D,L-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine.

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as quickly as possible. Dissection was complete 3–4 min after decapitation. The procedure was the same in the experimental and control groups. The indoles were not determined in olfactorius or cerebellum.

The compounds tested were given at the following time intervals before sacrifice: apomorphine—30 min, RO 4-4602—90 min, L-dopa—60 min, clonidine—60 min (75 min in experiments with L-dopa). Apomorphine and clonidine were injected subcutaneously as solutions in saline, RO 4-4602 and L-dopa were injected intraperitoneally as suspensions in a 3 per cent solution of Tween 80.

Statistical significance of the results was ascertained using the Student's *t*-test.

The following substances were used: clonidine hydrochloride [2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, Beohringer, Ingelheim]; apomorphine hydrochloride (Cefarm, Warszawa); L-dopa [L-(3,4-dihydroxyphenyl)-L-α-alanine, Reanal, Budapest]; RO 4-4602 [*N*-(D,L-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine, Hoffmann–La Roche, Basel]. The doses used refer to the forms listed here.

RESULTS

Clonidine. Given at a dose of 1 mg/kg clonidine did not alter the 5-HT or 5-HIAA level in the whole brain of rats and mice (Table 1).

TABLE 1. 5-HT AND 5-HIAA LEVELS IN THE BRAIN OF RATS AND MICE AFTER TREATMENT WITH CLONIDINE, APOMORPHINE OR CLONIDINE + APOMORPHINE

Group	Animal	Treatment (mg/kg)	5-HT level Wet wt of brain (ng/g)	(P)	5-HIAA level Wet wt of brain (ng/g)	(P)
I	Rat	—	637·0 ± 16·4	—	673·6 ± 53·5	—
II	Rat	Clonidine 1	635·2 ± 29·7	II/I N.S.	678·5 ± 78·8	II/I N.S.
III	Rat	Apomorphine 5	600·9 ± 34·8	III/I N.S.	1048·8 ± 64·6	III/I < 0·001
IV	Rat	Clonidine 1 + apomorphine 5	580·5 ± 22·4	IV/III N.S.	537·6 ± 63·4	IV/III < 0·001
V	Mouse	—	560·0 ± 16·3	—	580·1 ± 12·1	—
VI	Mouse	Clonidine 1	590·0 ± 18·1	VI/V N.S.	618·5 ± 37·4	VI/V N.S.
VII	Mouse	Apomorphine 5	567·5 ± 18·1	VII/V N.S.	747·0 ± 44·7	VII/V < 0·01
VIII	Mouse	Clonidine 1 + apomorphine 5	550·0 ± 31·2	VIII/VII N.S.	575·6 ± 44·9	VIII/VII < 0·02

Each value is the mean ±S.E.M. from eight to 10 animals. Animals were killed 30 min after apomorphine (s.c.) and 60 min after clonidine (s.c.). Statistical significance was calculated by the Student's *t*-test at $P < 0·05$.

N.S.—Not significant.

At doses of 0·1, 1·0 and 10 mg/kg clonidine did not change the 5-HT level in the rat brain cortex, striatum, thalamus and hippocampus (Table 2). The highest dose used elevated the 5-HT level in the hypothalamus and colliculi + tegmentum. In pons + medulla oblongata the 5-HT level was increased by each dose used (to 143·9, 125·3 or 131·0 per cent).

Clonidine and apomorphine. The results are shown in Table 1. In rats apomorphine (5 mg/kg) did not affect the brain 5-HT level but elevated the 5-HIAA level (to 174·6 per cent). Clonidine (1 mg/kg) given before apomorphine did not change the 5-HT

TABLE 2. 5-HT LEVELS IN DISCRETE REGIONS OF RAT BRAIN AFTER CLONIDINE

Brain region	5-HT levels (ng/g wet wt brain region) after: 0·9% NaCl	Clonidine 0·1 mg/kg	Clonidine 1 mg/kg	Clonidine 10 mg/kg
Cortex	241·1 ± 6·1	240·0 ± 6·3 N.S.	249·1 ± 7·0 N.S.	240·0 ± 8·3 N.S.
Striatum	554·6 ± 18·4	524·1 ± 14·3 N. S.	524·4 ± 30·0 N.S.	501·3 ± 32·6 N.S.
Thalamus	915·2 ± 70·9	1173·7 ± 120·4 N.S.	1057·5 ± 54·7 N.S.	924·6 ± 51·2 N.S.
Hypothalamus	1392·2 ± 55·4	1448·8 ± 169·0 N.S.	1455·0 ± 74·0 N.S.	1640·4 ± 82·8 P < 0·05
Hippocampus	777·1 ± 57·4	762·4 ± 55·1 N.S.	776·4 ± 42·9 N.S.	694·8 ± 45·5 N.S.
Colliculi + tegmentum	1181·0 ± 48·6	1246·3 ± 52·6 N.S.	1262·7 ± 93·7 N.S.	1386·6 ± 76·8 P < 0·05
Pons + medulla oblongata	584·7 ± 16·5	841·6 ± 33·5 P < 0·001	732·8 ± 32·9 P < 0·01	766·0 ± 16·0 P < 0·001

Each value is the mean ±S.E.M. from seven rats. Rats were killed 60 min after clonidine (s.c.). Statistical significance was calculated by the Student's *t*-test at P < 0·05 by comparison with the saline treated group.
N.S.—Not significant.

brain content, but counteracted the increase in the 5-HIAA level. Similar results were obtained in mice (Table 1).

Clonidine and L-dopa (together with RO 4-4602). The results are presented in Table 3. L-Dopa (200 mg/kg) given after RO 4-4602 (25 mg/kg) decreased the brain 5-HT level (to 41·5 per cent) and increased the brain 5-HIAA level (to 166·9 per cent). The 5-HT depletion and the 5-HIAA accumulation were both prevented by clonidine (1 mg/kg). RO 4-4602 given alone or with clonidine did not change the 5-HT and 5-HIAA levels (the data are not given in Table 3).

TABLE 3. 5-HT AND 5-HIAA LEVELS IN THE BRAIN OF RATS AFTER TREATMENT WITH CLONIDINE, L-DOPA (WITH RO 4–4602) OR CLONIDINE + L-DOPA (WITH RO 4–4602)

Group	Treatment (mg/kg)	5-HT level: Wet wt of brain (ng/g)	5-HT level: (P)	5-HIAA level: Wet wt of brain (ng/g)	5-HIAA level: (P)
I	—	639·6 ± 33·8	—	612·9 ± 33·7	—
II	Clonidine 1	775·6 ± 66·2	II/I N.S.	528·0 ± 54·4	II/I N.S.
III	RO 4-4602 25 + L-dopa 200	265·6 ± 20·3	III/I < 0·001	1023·0 ± 43·2	III/I < 0·001
IV	RO 4-4602 25 + clonidine 1 + L-dopa 200	403·8 ± 28·5	IV/III < 0·01	773·1 ± 65·1	IV/III < 0·01

Each value is the mean ± S.E.M. from eight to 10 rats. Rats were killed 90 min after RO 4-4602 (i.p.), 75 min after clonidine (s.c.) and 60 min after L-dopa (i.p.). Statistical significance was calculated by Student's *t*-test at P < 0·05.
N.S.—Not significant.

DISCUSSION

Although clonidine does not affect the whole brain levels of 5-HT and 5-HIAA in rats and mice it markedly elevates the 5-HT content of the pons + medulla oblongata region (a region rich in the 5-HT neurons[11]) and, to a lesser degree, of the hypothalamus and colliculi + tegmentum region of rat brain.

It has been shown previously that apomorphine increases the brain level of 5-HIAA, and increases or does not affect the brain 5-HT level, both in the whole brain of mice and rats and in discrete areas of rat brain.[12,13] The apomorphine-induced elevation of 5-HIAA level observed in the present investigation was counteracted by clonidine in both the animal species tested.

The findings that L-dopa decreases the 5-HT level and increases the 5-HIAA level in the brain, mainly owing to the release of 5-HT from the neurons[14–17] are confirmed by our results. In this case clonidine also antagonizes either the elevation of brain 5-HIAA content or the depression of 5-HT level.

The mechanism of the action of clonidine presented here is not clear. The decrease in the 5-HIAA level observed after combined treatment (apomorphine + clonidine or L-dopa + clonidine) suggests either the inhibition of 5-HT synthesis or the inhibition of MAO activity. The inhibition of 5-HT synthesis is improbable, as clonidine does not decrease the brain 5-HT level in normal or in apomorphine- or L-dopa-treated animals. The MAO inhibition is also unlikely, as after clonidine the 5-HT levels in normal and in apomorphine-treated animals and the 5-HIAA level in normal animals remained unchanged. The antagonistic action of clonidine against the L-dopa-induced changes in the brain levels of 5-HT and 5-HIAA suggests that clonidine inhibits the release of intraneuronally bound 5-HT and protects it from the metabolizing action of MAO. The apomorphine-induced changes in the brain concentrations of 5-HT and 5-HIAA are abolished by spiroperidol and pimozide, compounds which given alone do not change the level of either brain indole,[12,13] and so it could be suspected that these biochemical effects of apomorphine result from a secondary, and not a direct activation of 5-HT neurons. Regardless of the mechanism of activation of 5-HT neurons induced by apomorphine, it leads to increased synthesis and release of a neuromediator.[12,13] It seems therefore that in the experiments with apomorphine, as in the experiments with L-dopa, the protecting action of clonidine may result from the inhibition of 5-HT release from its stores.

It is interesting that the inhibitory effect of clonidine was observed also in the peripheral organs. It inhibited the secretion of noradrenaline and acetylcholine induced by electrical stimulation of adrenergic or cholinergic nerves respectively.[18]

Although the elucidation of the mechanism of clonidine action requires further investigation, the present results indicate that clonidine does affect 5-HT neurons. Therefore it does not seem to be an agent acting specifically on the NA neurons.

The question arises about the relevance of the action of clonidine on 5-HT neurons for its pharmacological effects and for its interaction with other agents, e.g. with apomorphine. The 5-HT neurons may inhibit and/or modify the hyperactivating effect of increased NA receptor activity on locomotion.[5] They are also thought to inhibit the apomorphine-induced aggression.[19] Therefore it may be supposed that the potentiation of the apomorphine-induced hypermotility by clonidine described in the literature[1,5,6] is related to its inhibiting effect on 5-HT release and not only to the stimulation of NA receptors.

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