POSSIBLE INVOLVEMENT OF A CENTRAL NORADRENERGIC SYSTEM IN AUTOMUTILATION INDUCED BY CLONIDINE IN MICE*

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Abstract—Automutilation induced by a single large dose of clonidine was potentiated by pretreatment with methamphetamine, caffeine and theophylline, while it was inhibited by acute administration of reserpine, α-methyl-p-tyrosine, phenoxybenzamine, phentolamine and chlorpromazine. t-Dopa, 5-hydroxytryptophan and p-chlorophenylalanine had no effect on this abnormal behavior. Biochemical studies on brain monoamines revealed that noradrenaline was markedly increased and dopamine slightly so, but 5-hydroxytryptamine was never changed by clonidine. These results suggest that a central noradrenergic system may be involved in automutilation induced by clonidine in mice.

Clonidine is an antihypertensive agent, acting centrally by inhibition of the sympathetic outflow (1, 2, 3) and it has been suggested that this drug stimulates central noradrenergic receptors (4, 5, 6). Clonidine easily passes through the blood brain barrier (7, 8). A marked sedative effect has been observed in man, and this observation prompted clinical trials with this drug in agitated mental patients (9). In experimental animals, clonidine has been shown to produce a variety of effects such as sedation in dogs and cats, and decrease in locomotor activity in mice at low dosage levels and it also exhibited sympathomimetic signs such as exophthalmos and horripilation (1, 10). Clonidine at large doses, induced aggressive behavior such as biting and attacking and also caused intense tremor in mice (11).

Previous investigations by the authors of such aggressive behavior induced by large doses of this drug indicated that the biting behavior was facilitated by the central noradrenergic mechanism (12). Clonidine was also found to induce automutilation in mice housed individually in the absence of objects to bite (13). The present investigation was undertaken to examine whether or not the noradrenergic mechanism played a major role in inducing automutilation as well.

MATERIALS AND METHODS

Male mice of ddN strain, weighing 18–22 g, were used. Clonidine suspended in a 0.5%

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carboxymethyl cellulose (CMC) was injected i.p. For other drugs, see the figures and tables. The animal was placed on a smooth surface table and each mouse was individually covered with a transparent glass beaker of 15 cm in diameter and the occurrence of automutilation was observed for a period of 1 hr following clonidine administration. Ten mice were used for each experiment.

Male mice of ddN strain, weighing 18-22 g, were used for the assay of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in the brain. The number of animals used in each experiment are indicated in the table. The mice were decapitated after i.p. administration of either clonidine or CMC. In each experiment for NA and DA assay, the brain (except the cerebellum and olfactory bulb) was homogenized in 6 ml of 0.4 N perchloric acid containing 1% EDTA-2Na and 0.1% sodium metabisulfite. The homogenate was centrifuged at 17,000 g at 5°C for 20 min. The supernatant fluid was adjusted to pH 8.3 with sodium hydroxide solution, 0.1 ml of 2M Tris buffer solution (pH 8.3) was added after which the preparation was transferred to a 10 ml glass-stoppered tube containing 100 mg activated A12O3. The tube was shaken for 5 min and the precipitated A12O3 was washed four times with redistilled water. The catecholamines were then eluted by vigorous shaking of A12O3 with 1 ml of 0.1 N HCl for 10 min. The supernatant was transferred to a 10 ml polyethylene centrifuge tube and spun at 12,000×g at 5°C for 10 min. A 0.5 ml aliquot of the clear sample was taken for analysis. NA and DA were determined by the trihydroxyindole method of Chang (14). In the 5-HT assay, the brain (except the cerebellum and olfactory bulb) was homogenized in 10 ml of 0.1 N HCl and 5-HT was determined by the method of Snyder et al. (15).

All experiments were performed at a room temperature of 22±1°C. The statistical significance of the results was determined by Student's t-test for biochemical studies and by the Fisher exact probability test for behavior observation.

RESULTS

Effects of various drugs on automutilation induced by clonidine

Pretreatment with either caffeine at doses of 10 and 20 mg/kg or theophylline at 20 mg/kg i.p. increased the incidence of automutilation induced by clonidine 50 mg/kg i.p., and the mice which showed this behavior, not only bit and cut off the digits of their own forelimb but also bit the thoracic area leaving a bleeding lesion. When the dose of caffeine or theophylline was increased up to 50 mg/kg i.p., the incidence of automutilation induced by clonidine 50 mg/kg i.p. was reduced. This seemed to be due to the toxic effect of the combination of these drugs at such high doses, because these mice showed no movement in the cage and occasionally exhibited clonic convulsion. Automutilation was not induced by clonidine alone at a dose of 20 mg/kg i.p., but was induced by the drug at the same dose in the mice pretreated with caffeine and theophylline at doses ranging from 20 to 70 mg/kg i.p. (Figs. 1 and 2).

Methamphetamine at doses of 0.5-5 mg/kg i.p. given before clonidine 50 mg/kg i.p., decreased the incidence of automutilation (Fig. 3). Such may be due to the toxic effect of
FIG. 1 Effects of caffeine on clonidine-induced automutilation in mice. Caffeine, dissolved in saline, was given 15 min before clonidine. *p = 0.05 significant difference vs. clonidine 20 mg/kg i.p.

![Graph showing effects of caffeine on clonidine-induced automutilation in mice.](image)

FIG. 2 Effects of theophylline on clonidine-induced automutilation in mice. Theophylline, dissolved in saline, was given 15 min before clonidine. *p = 0.05 significant difference vs. clonidine 20 mg/kg i.p.

![Graph showing effects of theophylline on clonidine-induced automutilation in mice.](image)

combination of these two drugs at such high doses. Most of the mice showed no movement in the cage, when methamphetamine 0.5 mg/kg i.p. was combined with clonidine 50 mg/kg i.p. In mice pretreated with methamphetamine 1 mg/kg i.p., clonic convulsion was induced by clonidine at a dose of 50 mg/kg i.p. Methamphetamine, 5 mg/kg, given i.p. before clonidine 50 mg/kg, induced clonic convulsion followed by death in most animals. A combination of clonidine 20 mg/kg i.p. and methamphetamine at doses of 5–20 mg/kg i.p., induced automutilation (Fig. 3), but the toxic effects of these two drugs were observed, and some of the mice developed clonic convulsions when clonidine was combined with methamphetamine 20 mg/kg i.p.

Reserpine, α-methyl-p-tyrosine (α-MT), phenoxybenzamine, phentolamine and chlor-
FIG. 3 Effects of methamphetamine on clonidine-induced automutilation in mice. Methamphetamine dissolved in saline, was given 30 min before clonidine. **p<0.01 significant difference vs. clonidine 20 mg/kg i.p.

TABLE 1 Effects of various drugs on automutilation induced by clonidine 50 mg/kg i.p.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg i.p.)</th>
<th>Incidence</th>
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</thead>
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<tr>
<td>Control</td>
<td>50</td>
<td>4/10</td>
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<tr>
<td>Reserpine</td>
<td>0.25</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0/10*</td>
</tr>
<tr>
<td>α-MT</td>
<td>50</td>
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<tr>
<td></td>
<td>100</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0/10*</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>1</td>
<td>3/10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>10</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>20</td>
<td>2/10</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.5</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0/10*</td>
</tr>
</tbody>
</table>

Control: clonidine 50 mg/kg i.p. *p<0.05 significant difference vs. clonidine 50 mg/kg i.p. Reserpine was given 18 hr, α-MT 16 hr, phenoxybenzamine 2 hr, phentolamine and chlorpromazine 30 min before clonidine 50 mg/kg i.p. Reserpine and α-MT were suspended in CMC to a concentration of 0.5%, phenoxybenzamine and chlorpromazine were dissolved in saline, while phentolamine was diluted in distilled water.

L-Dopa at doses of 50–300 mg/kg i.p. given 18 hr before, 5-hydroxytryptophan (5-HTP) at doses of 50–200 mg/kg i.p. given 1 hr before and p-chlorophenylalanine (PCPA) at doses
<table>
<thead>
<tr>
<th>monoamine</th>
<th>treatment</th>
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<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
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<tbody>
<tr>
<td>NA</td>
<td>CMC</td>
<td>4</td>
<td>0.61±0.01</td>
<td>0.61±0.03</td>
<td>0.57±0.03</td>
<td>0.58±0.03</td>
<td>0.67±0.03</td>
</tr>
<tr>
<td></td>
<td>clonidine</td>
<td>5</td>
<td>0.68±0.01***</td>
<td>0.76±0.02***</td>
<td>0.69±0.02**</td>
<td>0.66±0.03</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>DA</td>
<td>CMC</td>
<td>4</td>
<td>1.01±0.03</td>
<td>1.10±0.09</td>
<td>0.94±0.06</td>
<td>0.97±0.05</td>
<td>1.12±0.07</td>
</tr>
<tr>
<td></td>
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<td>1.14±0.01</td>
<td>1.11±0.03*</td>
<td>1.02±0.01</td>
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<td>5-HT</td>
<td>CMC</td>
<td>4</td>
<td>0.58±0.03</td>
<td>0.57±0.03</td>
<td>0.56±0.04</td>
<td>0.65±0.04</td>
<td>0.61±0.03</td>
</tr>
<tr>
<td></td>
<td>clonidine</td>
<td>5</td>
<td>0.61±0.02</td>
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<td>0.60±0.03</td>
<td>0.62±0.03</td>
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</table>

Each value indicates mean ± S.E. ng/g wet tissue

*: p<0.05,  **: p<0.02,  ***: p<0.01. significant difference vs. CMC control.
of 50–200 mg/kg i.p. given 24 hr before clonidine, showed no effect on the incidence of automutilation induced by clonidine 50 mg/kg i.p.

Changes in brain amine levels

Clonidine was given to mice at a dose of 50 mg/kg i.p. in all experiments for the assay of NA, DA and 5-HT. In the control group, a 0.5% CMC solution was given in a volume of 0.1 ml/100 g i.p. The brain levels of each amine were determined at various periods after drug administration as shown in Table 2.

The brain NA level significantly increased at 0.5, 1, and 2 hr after administration of clonidine. The DA level was slightly increased 2 hr after clonidine, but the 5-HT level showed no change throughout the entire course of the experiment (Table 2).

DISCUSSION

It has been found that large doses of clonidine induce aggressive behavior such as biting and attacking when the treated mice are housed in pairs or in groups (11). In our previous report on such type of aggressive behavior, we indicated that the central adrenergic mechanism might play a major role in inducing such biting behavior (12). We have also found that clonidine at doses over 50 mg/kg i.p. induces automutilation in mice placed individually on a smooth surface table in the absence of objects to bite (13).

In the present experiment, the effects of drugs affecting the noradrenergic system on automutilation induced by clonidine were studied. The drugs used to stimulate this system were theophylline, caffeine and methamphetamine, and those for blocking it were reserpine, α-MT, phenoxybenzamine, phentolamine and chlorpromazine.

It has been reported that repeated administration of high doses of theophylline induced automutilation in rats (16). In the present experiments, theophylline did not induce automutilation in mice when administered alone either acutely or chronically at high doses, but the occurrence of automutilation was markedly increased when this agent was combined with clonidine. Caffeine was also found to increase automutilation induced by clonidine. It has been suggested that both caffeine and theophylline activate the central noradrenergic system (17), and that caffeine causes a sensitization of central NA receptors (18). However, the incidence of automutilation was decreased when clonidine 50 mg/kg i.p. was combined with a dose of 50 mg/kg i.p. of either caffeine or theophylline. Such may be due to the toxic effect of these drugs combined at such high doses. Methamphetamine at doses of 0.5–5 mg/kg i.p., inhibited the automutilation induced by clonidine 50 mg/kg i.p. This inhibition may be due to toxic effects of these drugs combined at such high doses. Methamphetamine, which stimulates the noradrenergic system (19), induced automutilation when combined with clonidine 20 mg/kg i.p.

Depletion of NA by acute administration of reserpine and blocking of NA synthesis by α-MT resulted in inhibition of the noradrenergic system and the occurrence of automutilation with clonidine was decreased. Phenoxybenzamine and phentolamine, α-receptor blocking agents do penetrate the blood brain barrier (20, 21, 22). Chlorpromazine also has an α-receptor blocking action. Phenoxybenzamine and chlorpromazine inhibited automutilation
induced by clonidine, but phentolamine did not significantly suppress this behavior. Pretreatment with L-Dopa, 5-HTP and PCPA showed no effects on automutilation induced by clonidine.

The biochemical studies indicated that NA was mainly affected by clonidine; i.e. large doses of clonidine caused an increase in NA content with slight increase in DA but there was no change in 5-HT of the brain throughout the experiments. These results are in good agreement with the findings of Laverty and Taylor (23) in rats. The slight increase in DA level appeared at 2 hr after clonidine administration and during this period almost all mice were sedated and did not exhibit automutilation behavior. It is hardly likely that clonidine inhibits monoamine oxidase as the content of 5-HT was not changed by clonidine. It was also reported that clonidine increased the NA content in rat brain even at a much lower dosage (4, 24). Braestrup (25) showed that clonidine caused a decrease in MOPEG in the rat brain. It is therefore plausible that increase in the NA level induced by large doses of clonidine may be due to a decrease in the release of NA.

From these results of drug interaction and biochemical studies, it can be concluded that a central noradrenergic system is mainly involved in automutilation induced by large doses of clonidine.

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