The Difference in the Site of Actions of Tricyclic Antidepressants and Methamphetamine on the Duration of the Immobility in the Behavioral Despair Test

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Abstract—A single administration of tricyclic antidepressants reduced the duration of the immobility in the behavioral despair test. The escape-directed behavior of tricyclic antidepressant-treated rats was observed in a cylinder partially filled with water. In contrast, although methamphetamine also reduced the duration of the immobility, an increase in the general motor activity was shown in methamphetamine-treated rats. Tricyclic antidepressants injected into the medial amygdaloid nucleus, not into the nucleus accumbens, suppressed the duration of the immobility. Methamphetamine completely suppressed the duration of the immobility not only when injected into the medial amygdaloid nucleus, but also when injected into the nucleus accumbens. The present results suggest that in the rat behavioral despair test, the medial amygdaloid nucleus may play an important role in the selective reductive effect on the duration of the immobility.

Recently, Porsolt et al. (1–3) have devised a "behavioral despair test" for rats and mice. They described that animals forced to swim in a restricted space ceased attempts to escape and became immobile, and this immobility was reduced by the typical and atypical antidepressants. From these results, they concluded that this system serves as a useful model for the study of antidepressants. However, a lack of specificity in this screening of antidepressants has been pointed out (4–6). Psychostimulants, anticholinergic agents and antihistaminergic agents also significantly reduced the duration of the immobility. Kitada et al. (7) reported according to this specificity that the duration of the immobility only during the first 5 min or 10 min of the 30 min test was reduced by antidepressants. In contrast to the antidepressants, psychostimulants and anticholinergic agents reduced the duration of the immobility not only during the first 5 min, but also during the next 15–25 min in the 30 min test. Therefore, it was concluded that these agents have a different mechanism of action in the brain. Gorka et al. (8) reported that a unilateral lesion of the amygdala reduced the suppressive effect of the systemic injection of imipramine on the duration of the immobility in the behavioral despair test. However, there has been no report which has studied the brain site of the actions of tricyclic antidepressants and psychostimulants on the duration of the immobility in the behavioral despair test.

The present study was performed to clarify the difference in the site of actions of tricyclic antidepressants and methamphetamine on the duration of the immobility in the behavioral despair test using the micro-injection of the drugs into the limbic structures.

Materials and Methods

Animals: Male Wistar strain rats weighing 160–180 g were housed in an air-conditioned room at 22±1°C with a 12 hr light-dark schedule (lights on at 7:00). Food and water were given ad libitum during the experimental period.
Measurement of immobility: Rats were individually placed in vertical plexiglas cylinders (height: 40 cm, diameter: 18 cm) containing 15 cm of water maintained at 25°C. After the rats were placed in the water for 15 min, they were removed and allowed to dry for 15 min in a 30°C drying room. The next day, they were again put into the cylinder for 5 min after drug administration. A rat was judged to be immobile whenever it remained floating in the water, in an upright position, showing only the small amount of movement necessary to keep its head above water. The total duration of the immobility during 5 min was measured.

Surgery: Rats were anesthetized with pentobarbital-Na and the guidecannula, which was constructed of a stainless steel cannula having 0.7 mm outer diameter and 15 mm total length, was bilaterally implanted, according to the stereotaxic coordinates of König and Klippele (9), into the medial amygdaloid nucleus (anterior (A): 5.1, lateral (L): 3.5, horizontal (H): −3.2) and the nucleus accumbens (A: 8.6, L: 1.2, H: −1.0). The cannulae were fixed on the skull with dental cement together with two screws into the skull, according to the procedure of Watanabe et al. (10). The drug experiments were started from 7 days after the implantation of the cannulae.

Drug administration and procedure: Non-cannulated rats received a single injection of saline as a control or the following drugs: imipramine, amitriptyline, desipramine and methamphetamine. These drugs were injected and the test was conducted 1 hr later, at the peak time of the drug effect. In chronically cannulated rats, test drugs (desipramine, amitriptyline, imipramine and methamphetamine) dissolved in a 0.9% saline solution were injected 5 min before the test into both sides of the limbic structure in a volume of 2 μl each through a 0.35 mm diameter needle inserted into the guide cannulae in unrestrained and unanesthetized rats. Five min was the most effective time in this experiment. Control rats received a bilateral injection of an equal volume of the saline solution. We referred to the report of Watanabe et al. (10) for the doses of these drugs in our experiment.

Results

Effects of systemic injection of drugs on the duration of the immobility: After tricyclic antidepressants were injected intraperitoneally, the rats showed signs of sedation while in their home cage. When the rats were placed into the cylinder 60 min after the injection of tricyclic antidepressants, they initially exhibited escaping behavior such as diving, climbing and jumping. All of the rats showed these behaviors in the early period of the 5 min test. These behaviors were gradually decreased, and the general motor activity was mixed. After that, they gradually became immobile and were observed to remain immobile for approximately 60–75% of the 5 min test. As shown in Table 1, tricyclic antidepressants suppressed the duration of the immobility in comparison with the saline-injected rats. On the other hand, methamphetamine markedly facilitated the locomotor activity at a dose of 1 mg/kg i.p. and induced stereotyped licking at a dose of 10 mg/kg i.p. in the home cage. In addition, methamphetamine at 1 and 10 mg/kg completely suppressed the immobility (Table 1). All of the methamphetamine-injected rats showed a marked increase in general motor activity from the beginning of the 5 min test. They hardly showed the escaping behavior which
was induced by tricyclic antidepressants. Namely, methamphetamine-treated rats only swam about in the cylinder.

**Effects of drugs injected into the medial amygdaloid nucleus and the nucleus accumbens on the duration of the immobility:** The saline injected-rats, being injected in the medial amygdaloid nucleus and the nucleus accumbens, showed a tendency for suppression of the immobility in comparison with the systemically saline-injected rats. The effects of the drugs injected into the medial amygdaloid nucleus and the nucleus accumbens are shown in Figs. 1 and 2. Rats showed slight sedation in the home cage when tricyclic antidepressants were injected into the medial amygdaloid nucleus and the nucleus accumbens. Desipramine (20 μg) and imipramine (20 μg) injected into the medial amygdaloid nucleus suppressed the duration of the immobility in comparison with the saline-injected rats. Rats initially showed escaping behavior, which was the same as those in the systemic injection. However, desipramine and amitriptyline injected into the nucleus accumbens did not have any effect on the immobility in the behavioral despair test.

On the other hand, methamphetamine (10 μg) injected into the medial amygdaloid nucleus and the nucleus accumbens showed a slightly facilitated the locomotor activity in the home cage and completely suppressed the duration of the immobility (Figs. 1 and 2). The methamphetamine-injected rats showed a marked increase in general motor activity in the cylinder, which was the same as those in the systemic injection.

**Discussion**

In the behavioral despair test, a single systemic administration of tricyclic antidepressants and methamphetamine reduced the duration of the immobility. The tricyclic antidepressants and methamphetamine-injected rats increased an escape-directed behavior and general motor activity in the cylinder, respectively.

In another experiment, tricyclic antidepressants injected into the medial amygdaloid nucleus suppressed the duration of the immobility. However, tricyclic antidepressants did not have any effect on the duration of the immobility when they were injected into the nucleus accumbens. Methamphetamine completely suppressed the duration of the immobility not only when injected into the medial amygdaloid nucleus,

### Table 1. Effects of tricyclic antidepressants and methamphetamine on the duration of the immobility

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg, i.p.)</th>
<th>N</th>
<th>Duration of the immobility</th>
<th>% of respective control (mean±S.E.)</th>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>7.5</td>
<td>8</td>
<td>100.0±4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8</td>
<td>86.2±4.1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
<td>68.5±7.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>8</td>
<td>73.5±4.1***</td>
<td></td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>15</td>
<td>8</td>
<td>69.6±4.6***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
<td>70.2±5.4***</td>
<td></td>
</tr>
<tr>
<td>Desipramine</td>
<td>7.5</td>
<td>8</td>
<td>88.9±7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8</td>
<td>82.2±7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
<td>57.1±5.5***</td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>1</td>
<td>8</td>
<td>11.4±4.0***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>0 ± ***</td>
<td></td>
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</table>

Drugs were intraperitoneally injected 60 min before the behavioral despair test. Results are expressed as a % of the respective control. The mean duration (±S.E.) of the immobility of the saline-injected rats was 210.7±6.5 sec. (N=34) The significance of differences from the control was assessed statistically using the two-tailed Student's t-test (*P<0.05, **P<0.01, ***P<0.001).
Fig. 1. Effect of tricyclic antidepressants and methamphetamine injected into the medial amygdaloid nucleus on the duration of the immobility. Imipramine (20 μg/μl), desipramine (20 μg/μl), amitriptyline (20 μg/μl) and methamphetamine (10 μg/μl) were injected 5 min before the test into both sides of the medial amygdaloid nucleus in a volume of 2 μl through a 0.35 mm diameter needle inserted into the chronically implanted guide cannulae in unrestrained and unanesthetized rats. Each value is the mean for 10 animals. The significance of differences from the control was assessed statistically using the two-tailed Student’s t-test. *P<0.05, **P<0.01, ***P<0.001.

Fig. 2. Effect of tricyclic antidepressants and methamphetamine injected into the nucleus accumbens on the duration of the immobility. Desipramine (20 μg/μl), amitriptyline (20 μg/μl) and methamphetamine (10 μg/μl) were injected 5 min before the test into both sides of the nucleus accumbens in a volume of 2 μl. Each value is the mean for 10 animals. **P<0.01, ***P<0.001, by the two-tailed Student’s t-test.
but also when injected into the nucleus accumbens.

From the results described above, it is concluded that the amygdala is the site of the tricyclic antidepressant’s action in the behavioral despair test. This hypothesis is supported by the fact that a unilateral lesion of the amygdala significantly decreases the potency of imipramine in the behavioral despair test as regards to rats (8). Tricyclic antidepressants inhibit amygdaloid kindled seizures (11, 12) and after-discharges from the amygdala in rats (13). Moreover, Watanabe et al. (10) reported that particularly when tricyclic antidepressants were injected into the medial amygdaloid nucleus, they significantly suppressed the mouse-killing behavior (muricide) in olfactory bulbectomized rats, which is selectively blocked by the systemic injection of tricyclic antidepressants (14–16). It is considered that the amygdala may be the most important site of action of tricyclic antidepressants.

Escape-directed behavior such as diving, climbing and jumping and increased general motor activity was observed in the cylinder in the tricyclic antidepressants- and methamphetamine-treated rats, respectively. Therefore, it is assumed that tricyclic antidepressants reduced the duration of the immobility by prolonging the escape-directed behavior, and methamphetamine reduced it by increasing the general motor activity. Kitada et al. (7) also reported that the escape-directed behavior, which only appeared during the first 5 min, was prolonged by antidepressants. Furthermore, psychostimulants and anticholinergic drugs reduced the duration of the immobility by increasing the general motor activity. In fact, in the present experiments, methamphetamine markedly facilitated the locomotor activity and the stereotyped licking at a dose of 1 mg/kg and 10 mg/kg i.p. in the home cage, respectively. Methamphetamine injected into the medial amygdaloid nucleus and the nucleus accumbens also induced a slight facilitation of the locomotor activity in the home cage. It is reported that methamphetamine injected into the medial or anterior amygdaloid nucleus did induce a marked increase in exploratory behavior such as rearing (10). Therefore, it is conceivable that the reductive action of methamphetamine in the duration of the immobility in the behavioral despair test is based on non-specific action, perhaps its psychostimulating action.

The present results suggest that in the behavioral despair test, the medial amygdaloid nucleus may play an important role in the selective reductive effects by tricyclic antidepressants on the duration of the immobility.

References


