EFFECTS OF TRICYCLIC ANTIDEPRESSANTS ON TETRABENAZINE-INDUCED DEPLETION OF BRAIN MONOAMINES IN RATS. 2. DOPAMINE

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Abstract—We studied the effects of tricyclic antidepressants on the tetrabenazine (TB)-induced depletion of brain dopamine (DA) using rats. The test drugs were generally administered orally 3 hours before sacrifice and 2 mg/kg of TB or reserpine (RES) was administered subcutaneously 2 hours before sacrifice. The TB-induced DA depletion was enhanced by pretreatment with desmethylimipramine (DMI, 12.5–100 mg/kg), imipramine (12.5–100 mg/kg), chlorimipramine (25–100 mg/kg), amitriptyrine (100 mg/kg), maprotyrine (50 mg/kg) and chlorpromazine (5–20 mg/kg i.p.), while these drugs did not enhance RES-induced depletion. Observations to elucidate the action mechanism of antidepressant-induced enhancement were as follows. After TB administration, brain DA content was at the minimal level at 30 min after and on the way to recovery at 2 hours, but it approached the minimal level at 2 hours after RES administration. DMI pretreatment did not enhance the DA depletion at 0.5 hours after TB administration. In pargyline-pretreated rats, TB produced a decrease of brain DA with an increase of 3-methoxy-tyramine (3-MT), while RES showed only a slight effect on DA and 3-MT up to 2 hours. Amphetamine sulfate (20 mg/kg i.p.) slightly increased, while combinations with DMI decreased brain DA. These results suggest that tricyclic antidepressants inhibit DA reuptake from the synaptic cleft in vivo.

Tricyclic antidepressants inhibit norepinephrine (NE) or serotonin (5-HT) reuptake at the neuronal cell membrane and this effect is considered an important action mechanism of these drugs (1–4). These agents reportedly do not interfere with dopamine (DA) reuptake (4, 5). However, findings in recent studies suggest that antidepressants may interact with DA (6–8). We previously reported that tricyclic antidepressants enhance tetrabenazine (TB)-induced NE depletion, reflecting an inhibition of NE reuptake from the synaptosomal cleft (9). In these studies, we simultaneously determined brain DA and found that antidepressants also enhance the TB-induced DA depletion, reflecting an inhibition of DA reuptake.

MATERIALS AND METHODS

Seven-week-old male Sprague-Dawley rats were exsanginated, the brain quickly removed, and the hemisphere which had been placed in acid-butanol or 0.4 N perchloric acid was homogenized and centrifuged. DA content of the acid-butanol
extract was determined by the fluorimetric method of Chang (10) with slight modification and the 3-methoxytyramine (3-MT) content in the perchloric acid extract by the fluorimetric method of Karasawa et al. (11).

The drugs used were imipramine hydrochloride (IM, Tofranil® Inj.-Fujisawa), desmethylimipramine hydrochloride (Perfran® Inj.-Fujisawa), chlorimipramine hydrochloride (CI, Anafranil® Inj.-Fujisawa), maprotyrine hydrochloride (synthesized in the Shionogi Laboratories), amitriptyrine hydrochloride (AT), chlorpromazine hydrochloride (CPZ), DL-amphetamine sulfate (Zedrin® Takeda), tetrabenazine (TB, Rubigen® Inj.-Eisai), reserpine (Apoplon® Inj.-Daiichi) and pargyline hydrochloride (Regis). The doses except for that of amphetamine were calculated as those of the free bases. IM, DMI, CI, MT, and AT were administered p.o. as solutions or suspensions in 2.5% gum arabic. Amphetamine was administered p.o. as solutions in 2.5% gum arabic or i.p. as a 0.9% saline solution. Pargyline and CPZ were administered i.p. as 0.9% saline solutions. RES and diluted TB solution with 0.9% saline were administered s.c.

The significance of the difference between the means of the response was evaluated by Student’s t-test.

### RESULTS

1. Effects of IM and DMI on TB-induced depletion of brain DA content: Table 1 shows the effect of IM pretreatment on TB-induced depletion of brain DA. Pretreatment with 100 mg/kg of IM enhanced the DA depletion induced by TB 2 hr after TB administration and the degree of enhancement at 2 mg/kg of TB was larger than that at 10 mg/kg of TB. DMI pretreatment (50 mg/kg) also enhanced TB (2 mg/kg)-induced DA depletion at 1 to 3 hr after TB administration but showed little effect at 0.5 hr (Table 2).

2. Dose studies on the effects of several drugs on TB- and RES-induced depletion of brain DA content: Effects of several drugs on TB- and RES-induced depletion of the brain DA content were studied at 2 hr after administration of 2 mg/kg of TB or RES. As shown in Table 3, IM and DMI enhanced the TB-induced DA depletion in all doses tested (12.5–100 mg/kg). Enhancement of the TB-induced DA depletion was also observed with CI (25–100 mg/kg), AT (100 mg/kg), MT (50 mg/kg), and CPZ (5–20 mg/kg i.p.). Amphetamine partially suppressed the depletion at 10 mg/kg. These drugs had no effect on the RES-induced DA depletion.

| Treatment            | No. of rats | Dopamine (µg/g) |
|----------------------|-------------|-----------------|
| Control              | 5           | 0.90±0.02       |
| IM                   | 5           | 0.89±0.04       |
| TB 2                 | 5           | 0.27±0.01       |
| IM+TB 2              | 5           | 0.17±0.01†      |
| TB 10                | 5           | 0.14±0.00       |
| IM+TB 10             | 5           | 0.12±0.01†      |

Imipramine (100 mg/kg) was administered p.o. 1 hr before tetrabenazine and the rats were sacrificed 2 hr later. TB 2: Tetrabenazine 2 mg/kg s.c., TB 10: Tetrabenazine 10 mg/kg. Data represent the mean±S.E. †Significantly different from the corresponding tetrabenazine administration p<0.05. ‡Significantly different from the corresponding tetrabenazine administration p<0.01.
Effects of the highest doses of the tested drugs on the brain DA level were also studied 3 hr after drug administration. No change of the DA level was observed with IM, CI, TM and amphetamine. DMI and AT produced a slight decrease in the DA levels (data not shown).

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### Table 2. Effect of desmethylimipramine on tetrabenazine-induced depletion of brain dopamine in rats

| Time after tetrabenazine administration (hr) | Dopamine (µg/g) | Tetraimipramine | DMI + tetrabenazine |
|---------------------------------------------|----------------|-----------------|---------------------|
| Control 1.52±0.05 (6)                      |                |                 |                     |
| 2.0 0.62±0.01 (6)                           |                |                 |                     |
| Control 0.93±0.03 (4)                       |                |                 |                     |
| 0.5 0.18±0.01 (6)                           |                |                 |                     |
| 1.0 0.20±0.01 (6)                           |                |                 |                     |
| 3.0 0.38±0.01 (6)                           |                |                 |                     |

Desmethylimipramine (DMI, 50 mg/kg p.o.) was administered at 3 hr and tetrabenazine (2 mg/kg s.c.) at 0.5, 1.0, 2.0 or 3.0 hr before sacrifice. Data represent the mean±S.E. The number of rats is given in parentheses. †Significantly different from the corresponding tetrabenazine administration p<0.05. ‡Significantly different from the corresponding tetrabenazine administration p<0.01.

3. Comparative effects of TB and RES on brain DA content: To elucidate the differential effects of antidepressants on TB- and

### Fig. 1. Changes in the brain levels of dopamine after administration of tetrabenazine (TB, 2 mg/kg s.c.) and reserpine (RES, 2 mg/kg s.c.) in rats. Each point represents the mean of 6 determinations. Bars are S.E. of the mean.

### Fig. 2. Effects of tetrabenazine (TB) and reserpine (RES) on the brain levels of dopamine (DA) and 3-methoxytyramine (3-MT) in pargyline-pretreated rats. Pargyline (150 mg/kg i.p.) was administered 1 hr before TB (2 mg/kg s.c.) or RES (2 mg/kg s.c.). Bars are S.E. of the mean. Numbers in the columns indicate the number of determinations. *Significantly different from the corresponding saline administration p<0.01.
Table 3. Effects of several drugs on tetrabenazine-induced depletion of brain dopamine in rats

| Treatment (mg/kg)        | Dopamine (μg/g) |
|-------------------------|-----------------|
| Control                 | 1.52±0.05 (6)   |
| Tetrabenazine (TB)      | 0.62±0.01 (6)   |
| Desmethylimipramine     |                 |
| 12.5+TB                 | 0.53±0.03 (6)†  |
| 25  +TB                 | 0.47±0.02 (6)†  |
| 50  +TB                 | 0.43±0.03 (6)†  |
| 100 +TB                 | 0.43±0.03 (6)†  |
| Control                 | 1.26±0.01 (6)   |
| Tetrabenazine (TB)      | 0.55±0.02 (6)   |
| Imipramine              |                 |
| 12.5+TB                 | 0.45±0.03 (6)†  |
| 25  +TB                 | 0.48±0.02 (6)†  |
| 50  +TB                 | 0.46±0.02 (6)†  |
| 100 +TB                 | 0.47±0.02 (6)†  |
| Control                 | 0.81±0.04 (5)   |
| Tetrabenazine (TB)      | 0.32±0.02 (6)   |
| Chlorimipramine         |                 |
| 12.5+TB                 | 0.29±0.01 (6)   |
| 25  +TB                 | 0.28±0.01 (6)†  |
| 50  +TB                 | 0.25±0.01 (6)†  |
| 100 +TB                 | 0.25±0.01 (6)†  |
| Control                 | 0.90±0.01 (7)   |
| Tetrabenazine (TB)      | 0.27±0.01 (7)   |
| Amitriptyline           |                 |
| 12.5+TB                 | 0.28±0.01 (6)   |
| 25  +TB                 | 0.25±0.01 (6)   |
| 50  +TB                 | 0.25±0.01 (6)   |
| 100 +TB                 | 0.21±0.01 (7)†  |
| Control                 | 0.95±0.02 (6)   |
| Tetrabenazine (TB)      | 0.32±0.02 (5)   |
| Meprotoprine            |                 |
| 12.5+TB                 | 0.28±0.01 (6)   |
| 25  +TB                 | 0.29±0.01 (6)†  |
| 50  +TB                 | 0.25±0.01 (6)†  |
| Control                 | 1.02±0.03 (5)   |
| Tetrabenazine (TB)      | 0.37±0.01 (5)   |
| Chlorpromazine          |                 |
| 5  +TB                  | 0.23±0.00 (5)†  |
| 20 +TB                  | 0.21±0.01 (5)†  |
| Control                 | 1.05±0.04 (5)   |
| Tetrabenazine (TB)      | 0.24±0.01 (5)   |
| Amphetamine             |                 |
| 2 +TB                   | 0.23±0.01 (6)   |
| 10 +TB                  | 0.31±0.03 (5)†  |

Drugs (except chlorpromazine, i.p.) were administered p.o. 1 hr before tetrabenazine (2 mg/kg s.c.) and rats were sacrificed 2 hr later. Data represent the mean±S.E. The number of rats is given in parentheses. †, ‡ Significantly different from the corresponding depletor administration p<0.05. †† Significantly different from the corresponding depletor administration p<0.01.
DA content sharply decreased until a minimal level was reached at 0.5 hr, after which a gradual return to the control level was seen. However, after administration of RES, brain DA depletion decreased, approached the minimal level after 4 hr, then continued at this level.

In rats in which the brain monoamine oxidase (MAO) activity was almost completely inhibited by pretreatment with 150 mg/kg of pargyline, TB produced a decrease in the brain DA content with an increase of 3-MT, up to 3 hr. However, RES induced only a slight DA decrease and 3-MT increase, up to 2 hr, and produced changes at 4 hr which were smaller than those seen at 2 hr after TB administration (Fig. 2).

### Table 4. Effect of desmethylimipramine on amphetamine-induced change of brain dopamine level in rats

| Treatment (mg/kg) | No. of rats | Dopamine (\(\mu g/g\)) |
|------------------|-------------|------------------------|
| Control          | 6           | 0.93±0.02              |
| Amphetamine (A)  | 7           | 0.99±0.02*             |
| DMI 3.1+A        | 6           | 0.97±0.03              |
| DMI 6.3+A        | 6           | 1.00±0.02              |
| DMI 12.5+A       | 6           | 0.89±0.03†             |
| DMI 25.0+A       | 7           | 0.82±0.02‡             |

Desmethylylimipramine (DMI) was given p.o. 1 hr before i.p. administration of amphetamine (20 mg/kg) and rats were sacrificed 2 hr later. Data represent the mean±S.E. *Significantly different from amphetamine administration \(p<0.05\). †Significantly different from amphetamine \(p<0.01\). ‡Significantly different from control \(p<0.01\).

DISCUSSION

RES and TB, which are monoamine-depleting agents, exert similar biochemical effects on catecholamine (CA) stores by blocking the mechanism of amine storage granules (12). Although the action mechanisms of these two agents are generally considered identical, tricyclic antidepressants enhanced the TB-induced DA depletion but not the RES-induced depletion. In our experiments, the brain DA contents were usually determined 2 hr after the administration of 2 mg/kg of the depleting agents. At that time, the brain DA content was recovering from its TB-induced reduction to a minimal level. This shows that the mechanism of the amine storage granules had fairly recovered from the TB effect. In the case of RES, the amine storage mechanism remained blocked as the brain DA content approached a minimal level. TB caused a faster and more extensive decrease in DA and increase in 3-MT than RES in rats in which brain MAO activity was almost completely inhibited by pargyline treatment. This shows that TB has a more potent effect than RES on DA release to the synaptic cleft, up to 2 hr. These findings show that
the differential effects of TB and RES in antidepressant-pretreated rats were due to the differential degrees of their DA-releasing action to the synaptic cleft and the different duration of their action on the mechanism of the DA storage granules. This is supported by the finding that DMI produced no changes in the TB-induced DA depletion at 0.5 hr after TB administration, when TB had a fairly strong effect on the storage granules, as in the case of RES. These observations also suggest that enhancement of the TB-induced DA depletion with tricyclic antidepressants reflect their blockage of DA reuptake from the synaptic cleft.

Amphetamine produces an increased release of DA to the synaptic cleft (13, 14). This drug slightly increased the DA content in nontreated rats, but decreased these contents in DMI-pretreated rats, as was also found by other workers (15). These findings also support the idea that enhancement of TB-induced DA depletion reflects blockage of DA reuptake. DMI inhibits amphetamine metabolism and increases the brain amphetamine content (15–17). However, amphetamine increased the brain DA content, so the DMI-induced DA decrease could not be elucidated by assessing DMI-induced changes in amphetamine metabolism.

CPZ, an antipsychotic drug, also enhanced the TB-induced DA depletion and thus blocked DA reuptake. However, as CPZ is a dopaminergic blocking agent (18, 19), the possibility remains that the enhancement of TB-induced DA depletion increases with an increase in the release of DA.

Amphetamine reportedly does not affect the uptake of DA in vivo (4), therefore our observation that amphetamine did not enhance the TB-induced DA depletion also supports our proposal. This drug reduced TB-induced DA depletion, in a dose of 10 mg/kg.

We previously reported that tricyclic antidepressants enhanced the TB-induced NE depletion, thereby reflecting blockage of reuptake from the synaptic cleft (9). The present findings in the case of DA are similar to those for NE, showing that tricyclic antidepressants inhibit DA reuptake at doses similar to those related to NE reuptake inhibition.

We conclude that tricyclic antidepressants enhance the TB-induced DA depletion at 2 hr after administration of 2 mg/kg of TB, thereby reflecting the fairly selective inhibition of DA reuptake from the synaptic cleft.

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