EFFECT OF ZOTEPINE ON HEAD-TWITCH INDUCED BY L-5-HYDROXYTRYPTOPHAN, MESCALINE AND 2,5-DIMETHOXY-4-METHYLMAMPHTAMINE IN MICE AND RATS

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Abstract—The effect of zotepine, a new neuroleptic, on head-twitch induced by L-5-hydroxytryptophan (L-5HTP), mescaline and 2,5-dimethoxy-4-methylamphetamine (DOM) in mice and rats was compared with that of known neuroleptics and the serotonin receptor blocker cyproheptadine. Among the neuroleptics tested, zotepine and haloperidol produced potent inhibitory effects on head-twitch induced by these three drugs. The results indicate that zotepine has a potent anti-hallucinogenic effect.

Zotepine, 2-chloro-11-(2-dimethyl-aminoethoxy) dibenzo [b, f] thiepin, synthesized at the Research Laboratories of the Fujisawa Pharmaceutical Co. Ltd., is a new neuroleptic structurally different from known neuroleptics. Uchida et al. (1) have reported that zotepine exerts its neuroleptic effects mainly through anti-dopaminergic activity. Shimomura et al. (2) have shown that zotepine is a unique neuroleptic with potent central anti-serotonergic activity. L-5-hydroxytryptophan (L-5HTP), some hallucinogens and benzodiazepines can induce head-twitch in animals (3-8). Head-twitch is reported to be related to the action of serotonin, since this behaviour increased dose-dependently in tests with L-5HTP, and was inhibited by the anti-serotonergic drugs cyproheptadine and methysergide (3, 9). Corne and Pickering (4) have suggested that there is a good correlation between drug-induced hallucination in man and drug-induced head-twitch in animals, and some drugs which can antagonize hallucination in man can inhibit head-twitch induced by hallucinogens in animals.

It is therefore expected that zotepine can inhibit drug-induced head-twitch. The present study attempted to examine the inhibitory effect of zotepine on head-twitch induced by L-5HTP, mescaline and 2,5-dimethoxy-4-methylamphetamine (DOM) (10, 11), in comparison with that of known neuroleptics.

Materials and Methods

Male ddY mice (Shizuoka Agricultural Cooperative Association for Laboratories Animals), weighing 16-25 g, and female Wistar rats (Kuroda Animal Farm) weighing 200-250 g were used.

Drugs: Drugs used in the experiments were zotepine, chlorpromazine hydrochloride, and mescaline (synthesized in Fujisawa Labora-
haloperidol (Janssen), thioridazine hydrochloride (extracted from Melleril®, Sankyo), cyproheptadine hydrochloride (Nihon-Merck Banyu), L-5-hydroxytryptophan (L-5HTP) (Sigma), 2,5-dimethoxy-4-methylamphetamine (DOM) (supplied by the Ministry of Public Welfare of Japan).

Zotepine was dissolved in an equivalent amount of 1N hydrochloric acid and diluted to final concentration with saline. Haloperidol was dissolved in a minimum amount of 20% acetic acid and diluted with saline. L-5HTP was suspended in 0.5% methylcellulose aqueous solution. The other drugs were dissolved in saline. Mescaline and DOM were injected s.c., while the others were given i.p.

Measurement of head-twitch: A preliminary experiment was conducted with both kinds of test animals to determine the optimal time-interval and dose-levels of the three hallucinogens to induce head-twitch. Mice and rats were used in groups of 10 and 3 to 13, respectively.

Mice: Each drug dose was given to 5 animals, and two independent experiments were conducted. Mice were placed individually in transparent plastic cylinders to observe the number of head-twitches. Neuroleptics were given 30 min before dosing with 500 mg/kg L-5HTP, and the number of head-twitches was counted during 2 min at 20, 30 and 40 min after dosing with L-5HTP. The peak effect at any of the three time points was used to express the results. The experiment was performed at a temperature of 22–24°C.

Rats: Each drug dose was given to 3 to 13 animals. The rats were housed individually in transparent plastic cages placed in a sound proof box equipped with a fluorescent lamp to observe the number of head-twitches. Neuroleptics were given simultaneously with 50 mg/kg mescaline or 60 min before dosing with 2 mg/kg DOM, and the number of head-twitches was counted between 60 and 80 min after dosing with mescaline or during 20 min immediately after dosing with DOM. The experiment was performed at a temperature of 22–24°C.

Statistical analysis: The fifty % effective dose (ED50) was calculated according to the method of Litchfield and Wilcoxon.

Results

Effect of drugs on L-5HTP-induced head-twitch in mice: Figure 1 shows the time course of head-twitches induced by L-5HTP. L-5HTP was used in doses of 125, 250 and 500 mg/kg, and the number of head-twitches was counted during 2 min at 10 min intervals for 60 min after dosing with L-5HTP. The highest frequency of head-twitches was counted at 20, 30 and 40 min after dosing with 500 mg/kg L-5HTP. The peak effect at any of the three time points was used to express the results. The effect of the test drugs on L-5HTP-induced head-twitches is shown in Table 1. Zotepine in doses of 1.0 and 3.2 mg/kg produced a marked inhibition and at 10 mg/kg completely
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inhibited the L-5HTP-induced head-twitches. Zotepine, thioridazine and haloperidol markedly and completely inhibited the head-twitches at doses of less than 10 mg/kg. The serotonergic blocker cyproheptadine in doses of 10 mg/kg and over also markedly inhibited the head-twitches. According to the ED50 values, the order of potency was as follows: zotepine = haloperidol > chlorpromazine > cyproheptadine > thioridazine. Zotepine and haloperidol were almost equipotent, being about 3 times more potent than chlorpromazine.

Effect of drugs on mescaline-induced head-twitch in rats: The number of head-twitches during each 5 min was recorded for 2 min intervals at 20, 30 and 40 min after L-5HTP dosing, and the peak effect at any of the three time points was used to express the results. ED50 values were calculated according to the incidence of inhibition obtained as follows: Number of animals showing frequency of head-twitches less than half that of control/Number of animals used.

### Table 1. Effect of neuroleptics on head-twitch induced by L-5HTP (500 mg/kg, i.p.) in mice

| Drug      | Dose (mg/kg, i.p.) | Frequency of head-twitches (Mean±S.E.) | Incidence of inhibition (%) | ED50 (95% confidence limit) |
|-----------|--------------------|----------------------------------------|----------------------------|-----------------------------|
| Zotepine  | 0                  | 16.2±3.4                               | 0/10 (0)                   |                             |
|           | 0.1                | 14.4±2.6                               | 2/10 (20)                  |                             |
|           | 0.32               | 13.0±2.0                               | 3/10 (30)                  | 0.39                        |
|           | 1.0                | 4.4±1.6                                | 6/10 (80)                  | (0.19–0.80)                 |
|           | 3.2                | 0.3±0.3                                | 10/10 (100)                |                             |
|           | 10.0               | 0.0±0.0                                | 10/10 (100)                |                             |
| Chlorpromazine | 0            | 11.2±2.8                               | 0/10 (0)                   |                             |
|           | 0.32               | 13.4±2.5                               | 1/10 (10)                  | 1.10                        |
|           | 1.0                | 7.6±2.5                                | 6/10 (60)                  | (0.63–1.80)                 |
|           | 3.2                | 1.9±1.1                                | 6/10 (80)                  |                             |
|           | 10.0               | 0.0±0.0                                | 10/10 (100)                |                             |
| Thioridazine | 0              | 15.8±4.3                               | 0/10 (0)                   | 1.79                        |
|           | 1.0                | 9.1±3.1                                | 5/10 (50)                  | (0.72–4.47)                 |
|           | 3.2                | 8.4±1.8                                | 3/10 (30)                  |                             |
|           | 10.0               | 0.5±0.4                                | 10/10 (100)                |                             |
|           | 0                  | 10.0±2.7                               | 0/10 (0)                   |                             |
|           | 0.1                | 11.4±2.4                               | 3/10 (30)                  | 0.33                        |
| Haloperidol | 0.32             | 6.2±2.7                                | 5/10 (50)                  | (0.11–0.98)                 |
|           | 1.0                | 2.9±1.1                                | 7/10 (70)                  |                             |
|           | 3.2                | 2.8±1.6                                | 8/10 (80)                  |                             |
|           | 0                  | 13.1±2.1                               | 0/10 (0)                   |                             |
|           | 1.0                | 8.6±2.2                                | 4/10 (40)                  | 1.31                        |
| Cyproheptadine | 3.2          | 3.1±1.3                                | 8/10 (80)                  | (0.57–2.99)                 |
|           | 10.0               | 1.5±0.9                                | 9/10 (90)                  |                             |
|           | 32.0               | 0.5±0.3                                | 10/10 (100)                |                             |

Drugs were given i.p. 30 min before challenge with L-5HTP. The number of head-twitches was counted for 2 min intervals at 20, 30 and 40 min after L-5HTP dosing, and the peak effect at any of the three time points was used to express the results. ED50 values were calculated according to the incidence of inhibition obtained as follows: Number of animals showing frequency of head-twitches less than half that of control/Number of animals used.
50 mg/kg mescaline (average number of head-twitches (±S.E.) during 20 min, 37.4±9.0.) The effect of the drugs is shown in Table 2. Zotepine in doses of 0.1 mg/kg and over markedly inhibited mescaline-induced head-twitch. Zotepine and haloperidol and cyproheptadine were almost equipotent in this effect. According to the ED50 values, zotepine was approximately 50 and 100 times more potent than chlorpromazine and thioridazine, respectively.

Effect of drugs on DOM-induced head-twitch: The number of head-twitches for each 5 min was recorded for 60 min after dosing with DOM. Figure 3 shows that the effect peaked during the first 20 min after dosing with DOM in doses of 2 and 5 mg/kg. Since both doses gave almost the same response, an observation period of 20 min immediately after dosing with DOM in a dose of 2 mg/kg (average number of head-twitches (±S.E.) during 20 min, 40.4±6.4) was used to examine the effect of the drugs on head-twitch in rats (Table 3). Zotepine in doses of 0.25 mg/kg and over markedly inhibited DOM-induced head-twitch. According to the ED50 values, haloperidol was about 3 times more potent than zotepine, and zotepine was about 8 times more potent than chlorpromazine.

Discussion

In the present study, the effect of zotepine on head-twitch induced by L-5HTP and hallucinogens (mescaline and DOM) was studied. Head-twitch induced by these compounds has been used as a behavioural model for evaluating the central anti-serotonergic and anti-dopaminergic activity of drugs (3–8, 10, 11).

Zotepine was the most potent of the
Zotepine showed the strongest inhibitory effect on mescaline-induced head-twitch in rats, and it was about 50 times more potent than chlorpromazine. Haloperidol and cyproheptadine were nearly equipotent to zotepine. Zotepine and haloperidol also showed stronger effects than chlorpromazine in inhibiting DOM-induced head-twitch. We have previously reported that zotepine has a
central anti-dopamine effect comparable to that of chlorpromazine (1), but has a much more potent central anti-serotonin action (2). Conversely, haloperidol has been reported to have only a very weak central anti-serotonin effect (17), but found to have a central anti-dopamine effect 10-20 times stronger than zotepine and chlorpromazine (1). Although the central mechanism of head-twitch induced by mescaline and DOM has not been clarified, it has been suggested that the central serotonin system is involved in head-twitch induced by some hallucinogens (including mescaline and L-5HTP) (11, 15), and the present results also suggest the involvement of the central serotonin system since zotepine and cyproheptadine were both strongly inhibitory. In addition to the serotonin system, the central dopamine system may also be involved in head-twitch because haloperidol showed a strong inhibitory effect. Thus, both the central serotonergic and dopaminergic mechanisms may be involved in mescaline- and DOM-induced head-twitch in the same way as in L-5HTP-induced head-twitch.

Corne and Pickering (4) have suggested that there is a relationship between drug-induced head-twitch in animals (including mescaline-induced and L-5HTP-induced head-twitch) and drug-induced hallucination in man, and they have shown that clinically effective anti-hallucinatory drugs such as chlorpromazine and haloperidol antagonized head-twitch induced in animals by appropriate hallucinogens. Bobon et al. (18) have reported that haloperidol is the most effective among various neuroleptics in the treatment for hallucination and delusion in schizophrenics. The present results suggest that zotepine should provide an anti-hallucinatory effect in man comparable to that of haloperidol.

Table 3. Effect of neuroleptics on head-twitch induced by 2,5-dimethoxy-4-methylamphetamine (DOM) (2 mg/kg, s.c.) in rats

| Drug       | Dose (mg/kg, i.p.) | Frequency of head-twitches (Mean±S.E.) | Incidence of inhibition (%) | ED50 (95% confidence limit) |
|------------|--------------------|----------------------------------------|-----------------------------|----------------------------|
| Zotepine   | 0                  | 40.4±6.4                               | 0/8 (0)                     |                            |
|            | 0.05               | 36.0±11.2                              | 0/4 (0)                     |                            |
|            | 0.1                | 14.9±2.9                               | 3/8 (37.5)                  | 0.15                       |
|            | 0.25               | 8.1±2.0                                | 5/8 (62.5)                  | (0.07-0.33)                |
|            | 0.5                | 8.9±7.1                                | 7/8 (87.5)                  |                            |
| Chlorpromazine | 0.75              | 18.4±1.8                               | 4/8 (50)                    | 1.20                       |
|            | 1.0                | 12.6±4.6                               | 6/10 (60)                   | (0.84-1.79)                |
|            | 2.5                | 11.3±2.7                               | 8/12 (66.7)                 |                            |
|            | 5.0                | 1.8±1.0                                | 4/10 (100)                 |                            |
| Haloperidol | 0                  | 40.4±6.4                               | 0/8 (0)                     |                            |
|            | 0.01               | 29.4±5.6                               | 2/8 (25)                    |                            |
|            | 0.05               | 16.4±2.8                               | 3/8 (37.5)                  | 0.05                       |
|            | 0.1                | 6.1±1.5                                | 8/10 (80)                   | (0.02-0.13)                |
|            | 0.25               | 2.0±0.7                                 | 4/10 (100)                 |                            |

Drugs were given i.p. 60 min before DOM dosing. The effect of the drugs was measured during 20 min after challenge with DOM. ED50 values were calculated according to the incidence of inhibition obtained as follows: Number of animals showing less than 11 head-twitches (11 head-twitches correspond to the half of the least frequency of head-twitches of all the control group)/Number of animals used.
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