THE CENTRAL ANTI-SEROTONIN ACTIVITY OF ZOTEPINE, A NEW NEUROLEPTIC, IN RATS

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Abstract—2-Chloro-11-(2-dimethyl-aminoethoxy) dibenzo [b, f] thiepin (zotepine) is a new neuroleptic drug which is structurally different from known neuroleptics. Zotepine, chlorpromazine, properciazine, and cyproheptadine inhibited hyperthermia induced by dosing with fenfluramine in rats in a warm environment (26–28°C). Fenfluramine is known to induce hyperthermia by mediation of central serotonin. Zotepine had a 10 times or greater potency than chlorpromazine, properciazine and cyproheptadine in inhibiting the hyperthermia. Thioridazine did not inhibit the hyperthermia, whereas haloperidol accelerated the hyperthermia. Zotepine was also the most potent inhibitor of 3H-serotonin binding to rat cortical synaptosomes in vitro. However, cyproheptadine had the strongest anti-serotonin activity in rat fundus preparations, while zotepine and other neuroleptics showed the same order of potency. These results showed that zotepine is a unique neuroleptic with potent central anti-serotonin activity. The central anti-serotonin activity of zotepine is discussed in connection with its lesser extrapyramidal side effects in humans.
260 g were used.

Measurement of rectal temperature: Five to 10 animals were used per group. All experiments were performed at an ambient temperature of 26–28°C. Rats were housed five per cage and placed in a warm room at least 1 hr before the test. Rectal temperature was measured using a thermometer with a thermistor probe inserted about 5 cm deep into the rectum.

Effect of drugs on rectal temperature: In order to study the effect of drugs on rectal temperature, the rectal temperatures were measured 1, 2 and 3 hr after dosing with one of the drugs. In the case of fenfluramine-induced hyperthermia, the drugs were given 1 hr before dosing with fenfluramine, and the rectal temperatures were measured 1 and 2 hr thereafter.

$^3$H-serotonin binding assay: Rats were killed by decapitation and the brain was promptly removed. The cerebral cortex was dissected out and was homogenized in 10 volumes of 0.32 M sucrose solution with a Polytron homogenizer. After elimination of undisrupted tissue and nuclei by centrifugation at 750×g for 10 min, the supernatant was centrifuged at 10,000×g for 30 min. The resulting precipitate was suspended in 10 volumes of the original wet tissue weight of ice-cold water and maintained in an ice bath for 30 min with periodic agitation. This homogenate was centrifuged at 35,000×g for 20 min, and the precipitate was centrifuged in 40 volumes of the original wet tissue weight of 50 mM Tris-HCl buffer (pH 7.4). After incubation at 37°C for 10 min, the homogenate was centrifuged at 35,000×g for 20 min. The precipitate was washed, and resuspended in 50 mM Tris-HCl buffer (pH 7.4). This final resuspension (0.6–1.0 mg/ml) was used for the $^3$H-serotonin binding assay. 1.8 ml of this final resuspension solution was added to 0.1 ml of 40 nM $^3$H-serotonin (5-[1,2-$^3$H(N)]-hydroxytryptamine, specific activity of 30.1 Ci/mmol, New England Nuclear) and 0.1 ml of various concentrations of the test drugs. The samples were incubated at 37°C for 10 min. Non-specific binding of $^3$H-serotonin was determined by addition of 10 μM unlabelled serotonin. The samples were then filtered through a glass filter (Whatman GF/B) under vacuum. Radioactivity trapped in the filters was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, model 3255.

Serotonin-induced contraction of isolated stomach fundus preparation: Rats were fasted for 24 hr and exsanguinated from the carotid artery. The stomach was dissected out and placed in Tyrode’s solution of the following composition: NaCl 136.9, KCl 2.7, MgCl$_2$ 1.1, CaCl$_2$ 1.8, NaH$_2$PO$_4$ 0.4, NaHCO$_3$ 11.9, glucose 5.6 mM. The fundus of the stomach was cut into strips and suspended under a load of 0.5 g in a 25 ml organ bath containing Tyrode’s solution at 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. After a 20 min equilibration, 1.0×10$^{-7}$ g/ml serotonin was added to the bath at 10 min intervals until constant responses were obtained. Test drugs were added to the bath 3 min before the addition of serotonin. The contractions were recorded isometrically on a polygraph.

Drugs: Drugs used were zotepine (Fujisawa), chlorpromazine hydrochloride (Sigma), haloperidol (Janssen), thioridazine hydrochloride (extracted from Melleril®, Sankyo), propeliacazine, fenfluramine hydrochloride (synthesized in our Research Laboratories), cyproheptadine hydrochloride (Nihon Merck Banyu), and serotonin creatinine sulfate (Sigma). Zotepine and propeliacazine were dissolved in an equivalent amount of 1 N hydrochloric acid and diluted to final concentrations with saline. Haloperidol was dissolved in a minimum amount of 20% acetic acid and diluted with saline.
The other drugs were dissolved in saline. Test drugs were dosed p.o. and fenfluramine was dosed i.p.

**Statistical analysis:** The 50% effective dose (ED50) was calculated according to Litchfield and Wilcoxon, and statistical significance was analyzed according to the Student’s t-test or the Cochran-Cox method.

**RESULTS**

Effect of fenfluramine on rectal temperature in normal rats: Fenfluramine was used in a dose of 13 mg/kg, the dose recommended by Sulpizio et al. (3). As shown in Fig. 1, the mean rises of rectal temperature 1, 2 and 3 hr after dosing with fenfluramine were 0.9, 1.3 and 1.1 °C, respectively. In the control group, the rectal temperature showed no significant change.

Effect of drugs on fenfluramine-induced hyperthermia: Effects of drugs on fenfluramine-induced hyperthermia are shown in Table 1. The changes in the rectal temperature of rats were measured at 1 and 2 hr after dosing with fenfluramine. Zotepine in a dose of 0.032 mg/kg inhibited significantly, and in doses of 1.0 mg/kg and over, it inhibited markedly the fenfluramine-induced hyperthermia in rats. Chlorpromazine also inhibited markedly the hyperthermia in doses of 1.0 mg/kg and over. Propericiazine and cyproheptadine inhibited significantly the hyperthermia in doses of 1.0 and 3.2 mg/kg and 3.2 and 10 mg/kg, respectively. According to ID50, zotepine was about 10 times and over more potent than chlorpromazine, propericiazine and cyproheptadine, whereas thioridazine had no effect. Haloperidol, on the contrary, in doses...
Table 1. Effects of drugs on the hyperthermia induced by fenfluramine in rats

| Drug      | Dose (mg/kg) | Rectal temperature |          |          |
|-----------|--------------|---------------------|----------|----------|
|           |              |                     | 60 min   | 120 min  |
|           |              | Change (°C)         | Inhibition (%) | Change (°C) | Inhibition (%) |
| Zotepine  | 0            | +1.02±0.04          | 100      | +1.47±0.10 | 17.0 |
|           | 0.01         | -1.15±0.14          | -12.7    | +1.22±0.08 | 27.2 |
|           | 0.032        | +0.80±0.08          | 21.6     | +0.17±0.16 | 72.8 |
|           | 0.1          | -0.33±0.10**        | 67.6     | -0.40±0.13*** | 73.5 |
|           | 0.32         | +0.16±0.11**        | 84.3     | +0.39±0.11** | 100 |
|           | 1.0          | -0.24±0.09**        | 100      | -0.07±0.10** | 100 |
| Chlorpromazine | 0        | +1.14±0.22          | 28.0     | +0.98±0.20 | 27.9 |
|           | 0.32         | +0.82±0.20          | 100      | +0.10±0.23** | 92.6 |
|           | 1.0          | -0.18±0.17**        | 100      | -0.19±0.12** | 100 |
|           | 3.2          | +0.02±0.14**        | 100      | +0.03±0.09** | 98.4 |
| Properiazine | 0        | +1.15±0.14          | 16.5     | +1.46±0.18 | 21.0 |
|           | 0.32         | +0.96±0.10          | 33.9     | -1.30±0.20* | 30.1 |
|           | 1.0          | +0.76±0.17          | 100      | +0.03±0.09** | 98.4 |
|           | 3.2          | -0.43±0.08**        | 100      | -0.19±0.12** | 100 |
| Thioridazine | 0        | +1.10±0.12          | 8.2      | +1.36±0.13 | 21.0 |
|           | 0.32         | +0.94±0.11          | 14.5     | +1.35±0.18 | 21.5 |
|           | 1.0          | +0.91±0.18          | 17.3     | +1.30±0.18 | 30.1 |
|           | 3.2          | +0.78±0.14          | 29.1     | +1.20±0.17 | 98.4 |
| Haloperidol | 0        | +1.14±0.14          | -16.5    | +1.29±0.10 | 10.0 |
|           | 0.32         | +1.61±0.19**        | -66.0    | +1.80±0.15** | -47.5 |
|           | 1.0          | +2.10±0.19**        | -116.5   | +2.23±0.16** | -82.8 |
|           | 3.2          | +2.66±0.21**        | -174.2   | +2.48±0.12** | -103.0 |
| Cyproheptadine | 0        | +1.22±0.15          | 14.5     | +1.22±0.15 | 10.0 |
|           | 0.32         | -1.31±0.24          | -7.4     | +1.40±0.19 | 13.0 |
|           | 1.0          | +1.16±0.20          | 4.9      | +1.34±0.18 | 18.8 |
|           | 3.2          | +0.54±0.25**        | 55.7     | +0.61±0.19** | 62.1 |
|           | 10.0         | -0.12±0.12**        | 100      | -0.16±0.07* | 100 |

| ED50 (mg/kg) | 0.078 (0.039–0.157) | 0.065 (0.025–0.172) |
|--------------|----------------------|---------------------|
| Chlorpromazine | 0.32–1.0             | Ca. 0.6             |
| Properiazine  | 1.70 (0.464–6.24)    | 0.747 (0.371–1.51)  |
| Thioridazine  | 3.2                  | 3.2                 |
| Haloperidol   | 2.81 (1.69–4.67)     | 2.02 (1.11–3.68)    |

Drugs were given p.o. 1 hr before dosing with fenfluramine. Fenfluramine was given i.p. in a dose of 13 mg/kg. Control group (0 mg/kg) was given saline instead of a test drug. Each value with standard error represents changes in the rectal temperature 1 and 2 hr after dosing with fenfluramine. Each group included 10 animals. *P<0.05, **P<0.01: significantly different from the corresponding control group.
of 0.32 mg/kg and over produced a further increase of the rectal temperature of the fenfluramine-treated rats.

Effect of drugs on rectal temperature in intact rats: To determine whether the drugs inhibited the hyperthermia, specifically antagonizing the effect of fenfluramine, the effects of the drugs on the rectal temperature of intact rats were studied. The results are given in Fig. 2. Zotepine in doses of 0.1 and 1.0 mg/kg, chlorpromazine in a dose of 1.0 mg/kg, propericiazine in a dose of 3.2 mg/kg, cyproheptadine in a dose of 10 mg/kg, thioridazine in a dose of 3.2 mg/kg, and haloperidol in doses of 1.0 and 3.2 mg/kg had no effects on the rectal temperature of intact rats.

Effect of drugs on \(^3\)H-serotonin binding:

The amount of specific serotonin binding on the rat cerebral cortex membrane was 60±10 fmole/mg protein. Various neuroleptics caused dose-dependent displacement of radioactive serotonin binding to the cortex membrane. The results are shown in Table 2. The inhibitory effects of drugs on \(^3\)H-serotonin binding (IC50) were as follows: Zotepine = 2.3×10⁻⁷, chlorpromazine = 2.1×10⁻⁶, propericiazine = 2.9×10⁻⁶, thioridazine = 2.9×10⁻⁶, haloperidol = 1.7×10⁻⁶ and cyproheptadine = 5.0×10⁻⁶ M.

Inhibition of contraction of isolated stomach fundus by test drugs: The results are given in Fig. 3. The addition of any one of the drugs tested to the bath fluid inhibited the serotonin-induced contraction of the isolated stomach fundus. The results are presented as percent of control binding. The dose-response

| Drug         | M  | \(^3\)H-5HT bound % | IC50 (μM) |
|--------------|----|---------------------|-----------|
| Zotepine     | 10⁻⁶ | 70±1.9              |           |
|              | 10⁻⁷ | 56±3.1              | 0.23      |
|              | 10⁻⁶ | 43±2.3              |           |
|              | 10⁻⁵ | 23±2.3              |           |
| Chlorpromazine| 10⁻⁸ | 98±7.9              |           |
| Propericiazine| 10⁻⁷ | 86±4.4              | 2.1       |
|              | 10⁻⁶ | 61±9.2              |           |
|              | 10⁻⁵ | 28±1.2              |           |
| Thioridazine | 10⁻⁷ | 83±6.6              | 2.9       |
|              | 10⁻⁶ | 66±3.8              |           |
|              | 10⁻⁵ | 39±3.1              |           |
|              | 10⁻⁴ | 10±1.7              |           |
| Haloperidol  | 10⁻⁷ | 83±7.9              | 2.9       |
|              | 10⁻⁶ | 60±8.0              |           |
|              | 10⁻⁵ | 43±7.2              |           |
|              | 10⁻⁴ | 11±2.9              |           |
| Cyproheptadine| 10⁻⁷ | 83±7.9              | 1.7       |
|              | 10⁻⁶ | 61±5.2              |           |
|              | 10⁻⁵ | 28±3.2              |           |
|              | 10⁻⁴ | 7±5.4               |           |
|              | 10⁻³ | 92±5.6              | 5.0       |
|              | 10⁻² | 78±8.2              |           |
|              | 10⁻¹ | 62±7.5              |           |
|              | 10⁰  | 46±12.6             |           |

Each value represents the mean±S.E.M. of duplicate determinations from three separate experiments and is expressed as percent of the control binding measured in the absence of added drug.
Fig. 3. Inhibitory effect of drugs on the serotonin-induced contraction in the stomach strip of rat. Test drugs were added to the bath 3 min before the addition of serotonin 1.0×10^{-7}g/ml. Each point represents the mean of 3 experiments. The vertical lines indicate the standard errors. O: zotepine, □: chlorpromazine, ●: propericiazine, ▲: thioridazine, Δ: haloperidol, ■: cyproheptadine.

The curve of zotepine was not parallel with those of the other drugs. Among the drugs tested, cyproheptadine showed the strongest inhibition of contraction of the isolated stomach fundus and was about 20 times more active than zotepine. Zotepine was about five times more active than chlorpromazine, propericiazine, thioridazine and haloperidol. Chlorpromazine, propericiazine, thioridazine and haloperidol had almost the same activity.

DISCUSSION

The present study has confirmed that fenfluramine produces hyperthermia in rats in a warm environment (26–28°C). Fenfluramine is structurally similar to amphetamine. Fenfluramine reportedly releases serotonin and inhibits uptake of serotonin in the serotonergic nerves of the brain (4, 5). Therefore, the hyperthermic effect of fenfluramine depends on a serotonin mechanism rather than on the dopamine mechanism on which amphetamine depends. Sulpizio et al. (3) have suggested that the antagonism of fenfluramine-induced hyperthermia is a sensitive measure of central anti-serotonin activity. The present results showed that zotepine, chlorpromazine, propericiazine, and cyproheptadine inhibited fenfluramine-induced hyperthermia in rats. The rank order of potency was as follows: zotepine > chlorpromazine > propericiazine > cyproheptadine. Zotepine was about 10 times more potent than chlorpromazine. Thioridazine could not inhibit the hyperthermia, and haloperidol enhanced it.

Nelson et al. (7) have reported that there are almost no differences in the number of binding sites and the binding affinity for 3H-serotonin between synaptosomes from the cortex, hypothalamus, thalamus and striatum. Because rather large amounts of synaptosomes were needed to study the effects of drugs on the 3H-serotonin binding, the cerebral cortical synaptosomes were used in this study. Zotepine was almost the most potent among the drugs tested in inhibiting 3H-serotonin binding to cortical synaptosomes. The order of inhibiting activity was as follows: zotepine > haloperidol > chlorpromazine > propericiazine = thioridazine > cyproheptadine. Zotepine was about 10 times more potent than chlorpromazine in inhibiting the 3H-serotonin bind-
ing to cortical synaptosomes. Thus, the order of potency of zotepine, chlorpromazine, properic Diazine and cyproheptadine in inhibiting both fenfluramine-induced hyperthermia and $^3$H-serotonin binding to cortical synaptosomes were parallel. In spite of their potent inhibiting effects on $^3$H-serotonin binding, thioridazine and haloperidol did not inhibit the fenfluramine-induced hyperthermia as described above. The mechanism of this discrepancy was not evident in this study. Lai et al. (8) have also reported that thioridazine was weakly active in blocking the twitch of mylohyideus muscle induced in rats by quipazine a serotonin agonist and was highly active in inhibiting $^3$H-serotonin binding to cortical synaptosomes; whereas zotepine, clozapine, cyproheptadine and haloperidol showed similar effects in both systems. Several authors have reported that there are many kinds of serotonin receptors in the brain (9-11). Peroutka et al. (11) have recently shown that the cerebral cortex in rats has two distinct serotonin receptors, 5-HT$_1$ and 5-HT$_2$. There is the possibility that the discrepancy in pharmacological and biochemical effects of thioridazine and haloperidol depends on the various serotonin receptors in the brain. Further study is needed to determine the reason for this discrepancy. In the rat fundus preparations, we did not study the effects of neuroleptics under various concentrations of serotonin thus the dose-response curves show the total effects of the neuroleptics including non-specific muscle relaxant activity in addition to the competitive antagonism at receptor sites and each dose-response curve is not parallel with one another. However the results can be interpreted to suggest the rank order of anti-serotonergic potency of drugs used in the peripheral organs. It is interesting that zotepine which showed clearly stronger anti-serotonergic activity than cyproheptadine in the two central preparations had a clearly weaker anti-serotonergic activity in the peripheral preparation though we have no explanation for this phenomenon.

Our previous study (1) has shown that zotepine has the same anti-dopamine activity as chlorpromazine in rats and dogs. Extrapyramidal symptoms, side effects of neuroleptics are thought to be induced by the anti-dopamine activity of neuroleptics in the extrapyramidal system (12, 13). Anticholinergic activity is well known to antagonize the induction of extrapyramidal symptoms (14, 15). On the other hand, Kelly and Naylor (16) have postulated that the balance between dopamine and serotonin is related to the induction of extrapyramidal symptoms. Sulpizio et al. (3) have described that clozapine, which produces fewer extrapyramidal symptoms, was a potent blocker of fenfluramine-induced hyperthermia in rats, and they suggested that the lower incidence of extrapyramidal symptoms by clozapine in humans can be ascribed to its potent central anti-serotonin activity. Thus, although the role of serotonin in schizophrenia has not been established, a central anti-serotonin activity can be expected to decrease the incidence of extrapyramidal side effects.

In summary the present results show that zotepine is a unique neuroleptic in view of its potent central anti-serotonin activity and may cause fewer extrapyramidal symptoms in man.

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