Effect of Isofloxythepin, a Novel Neuroleptic, on Hippocampal Stimulation-Induced Wet-Dog Shaking in the Rat

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Abstract—(±)Isofloxythepin (0.32–3.2 mg/kg, i.p.) significantly inhibited in a dose-dependent manner wet-dog shaking (WDS) induced by electrical stimulation of the rat hippocampus. In addition, both optical isomers of isofloxythepin inhibited WDS, with the (−)-isomer being almost 3 times more potent than the (+)-isomer. Other neuroleptics such as haloperidol, chlorpromazine, zotepine and sulpiride also reduced significantly the number of WDS. The inhibitory potency of haloperidol was comparable to that of (±)isofloxythepin, which was approximately 3 times more potent than that of chlorpromazine or zotepine. Sulpiride suppressed significantly WDS only at the high dose of 100 mg/kg. None of the drugs affected hippocampal afterdischarge. Inhibition of WDS produced by (±)isofloxythepin or haloperidol was antagonized by pretreatment with a dopamine receptor agonist, lisuride. The present results indicate that isofloxythepin shares with other neuroleptics an inhibitory effect on WDS; dopaminergic blocking action appears to be important in the inhibition of WDS induced by hippocampal stimulation.

Isofloxythepin, 3-fluoro-8-isopropyl-10-\[4-(2-hydroxyethyl) piperazino\]-10,11-dihydrodibenzo(b,f)thiepin, is a newly synthesized neuroleptic drug, that has been shown to inhibit apomorphine-induced stereotyped behavior (1, 2). Isofloxythepin as well as other neuroleptics such as haloperidol and zotepine causes the elevation of serum prolactin level in rats (3, 4), which might be due to the blockade of dopamine receptors in the pituitary. Isofloxythepin has also been shown to increase striatal dopamine turnover in rats (3, 5), which presumably reflects the blockade of dopaminergic transmission in the striatum. Recently, Lau and Runice (1) indicated that isofloxythepin has a blocking action on the rat striatal dopamine receptors, using the \[^{[3]}H\] spiperone binding technique.

Wet-dog shaking (WDS) is a characteristic behavior observed in morphine-dependent rats after abrupt morphine withdrawal or after the administration of a narcotic antagonist (6). WDS can also be induced by electrical stimulation of the hippocampus (7–10), the septum (11) or the fornix (12). Recently, it has been shown that the hippocampal stimulation-induced WDS is significantly and dose-dependently inhibited by morphine (7, 9) and by representative neuroleptic drugs such as chlorpromazine and haloperidol (7, 8). These results indicate that central dopaminergic mechanisms may be important in WDS elicited by hippocampal stimulation because neuroleptics directly and morphine indirectly inhibit the dopaminergic function.

In this study, we investigated the effects of isofloxythepin on WDS induced by hippocampal stimulation and examined whether neuroleptic drugs in general have an inhibitory action on WDS.

Materials and Methods

Animals: The animals used were male Wistar rats (body weight: 260–300 g at the time of surgery) obtained from Shizuoka Laboratory Animal Center. The rats were housed in a room maintained at a temperature of 22±1°C with a 12 hr light-dark cycle (light period: 07:00–19:00). Food and water were
available ad libitum throughout the experimental period.

**Electrode implantation and experimental procedures:** Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and fixed in a stereotaxic instrument. Stainless steel bipolar electrodes (diameter: 0.2 mm, uninsulated length: 0.5 mm, interelectrode distance: 0.5 mm) were chronically implanted bilaterally in the dorsal hippocampus (anterior: 3.2 mm, lateral: 2.8 mm, horizontal: 3.0 mm) according to the rat brain atlas of Paxinos and Watson (13). Following implantation of the chronic electrodes, at least one week was allowed for recovery from surgery before starting the experiment.

Rats were placed in a transparent plastic box (30×25×30 cm) containing wood shavings and allowed to adapt to the new environment for 5–10 min prior to brain stimulation. The hippocampus was stimulated for 5 sec with a square wave pulse (60 Hz in frequency, 1.0 msec in duration, bipolar). One hippocampal electrode was used for stimulation and the other, for recording afterdischarge (AD). First, the threshold for inducing AD was determined, starting from an ineffective current of 30 μA. The stimulus intensity was increased in 10 μA increments until AD was induced. The initial stimulation intensity which induced AD was regarded as the threshold for AD. After the AD threshold was determined, electrical stimulation was performed at this intensity. The number of WDS was counted by an observer and simultaneously electrical responses from the dorsal hippocampus were recorded on a polygraph (Nihon Kohden) during the 180 sec period following the stimulation. The drug tests were carried out 5–6 hr after the measurement of control values.

**Drugs:** The following drugs were used in this study: (±)isofloxythepin methanesulfonate and its optical isomers, (+) and (−) isofloxythepin methanesulfonate (Showa Denko), haloperidol (Serenace Injection, Dainippon), chlorpromazine (Contomin Injection, Yoshitomi), zotepine (Fujisawa), sulpiride (Dogmatyl Injection, Fujisawa) and lisuride hydrogen maleate (Nihon Schering). Isofloxythepin and lisuride were dissolved in distilled water. Zotepine was dissolved in 0.1 N HCl, and the pH was adjusted to 4–5 with NaOH. Drugs were administered i.p. in a volume of 0.1 ml per 100 g of body weight, 1 hr before hippocampal stimulation. The pre-treatment with lisuride in the antagonism test was performed 10 min prior to drug injection.

**Statistical analysis:** The significance of the drug effect on the number of WDS and AD duration was determined using Student’s *t*-test or the Cochran-Cox test.

**Results**

Electrical stimulation of the dorsal hippocampus with an intensity above the AD threshold (mean±S.E.: 51.4±5.0 μA) induced a high frequency of WDS (31.8±2.6/3 min), which was associated with the occurrence of hippocampal AD. WDS was never induced by stimulation below this threshold. As shown in Fig. 1, (±)isofloxythepin (0.32–3.2 mg/kg, i.p.) caused a dose-dependent reduction in the number of WDS induced by hippocampal stimulation. The appearance of WDS was almost completely abolished when 3.2 mg/kg of (±)isofloxythepin was injected. However, at this dose, all animals showed severe sedation and ataxia. The (−)-isomer of isofloxythepin, at doses of 0.32 and 1.0 mg/kg, also markedly inhibited hippocampal stimulation-induced WDS. WDS was also significantly inhibited by (+)isofloxythepin at 1.0 mg/kg, but not by 0.32 mg/kg. The inhibitory effect of (−)isofloxythepin on WDS was almost 3 times more potent than that of its (+)-isomer. The duration of hippocampal AD was not significantly affected either by the racemic isofloxythepin or by its optical isomers (Table 1).

Figure 2 shows the effects of other neuroleptic drugs on hippocampal stimulation-induced WDS. All four neuroleptics used in this experiment produced a significant and dose-dependent inhibition of WDS. Haloperidol was almost equipotent to (±)isofloxythepin, which was approximately 3 times more potent than chlorpromazine and zotepine. Haloperidol, at a dose of 1.8 mg/kg, caused severe sedation and ataxia. Sulpiride suppressed significantly the appearance of WDS only at a high dose of 100 mg/kg. None of the drugs had an effect on hippocampal AD
Fig. 1. Effects of (+)-isofloxythepin (IFT) and its optical isomers on wet-dog shaking (WDS) induced by electrical stimulation of the rat hippocampus. IFT was injected i.p. 1 hr before hippocampal stimulation for 5 sec with a square wave pulse (60 Hz, 1.0 msec). The number of WDS was expressed as a percentage of the control values (Pre-drug) for each animal. Each value is the mean±S.E. for 5–10 animals. The number of WDS in the saline-treated group was 29.4±3.0/3 min. Significant differences from the saline group or between optical isomers were determined using Student's t-test or the Cochran-Cox test. *P<0.05, **P<0.01, ***P<0.001.

Table 1. Effects of isofloxythepin, haloperidol, chlorpromazine, zotepine, sulpiride and lisuride on afterdischarge induced by hippocampal stimulation in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>mg/kg i.p.</th>
<th>n</th>
<th>Afterdischarge duration (sec)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-drug</td>
</tr>
<tr>
<td>Saline</td>
<td>—</td>
<td>9</td>
<td>37.1±2.5</td>
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<tr>
<td>(+)-IFT</td>
<td>0.32</td>
<td>5</td>
<td>47.4±8.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6</td>
<td>43.3±6.3</td>
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<tr>
<td></td>
<td>3.2</td>
<td>6</td>
<td>49.7±8.1</td>
</tr>
<tr>
<td>(+)-IFT</td>
<td>0.32</td>
<td>5</td>
<td>42.8±5.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5</td>
<td>38.0±5.5</td>
</tr>
<tr>
<td>(-)-IFT</td>
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<td>5</td>
<td>38.4±2.7</td>
</tr>
<tr>
<td></td>
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</tr>
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<td></td>
<td>1.8</td>
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<td></td>
<td>100.0</td>
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<tr>
<td>Lisuride</td>
<td>0.32</td>
<td>8</td>
<td>39.8±5.5</td>
</tr>
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</table>

Animals were first stimulated before drug administration (Pre-drug). They were stimulated again, 5–6 hr later, following drug injection (Post-drug).
When administered alone at a dose of 0.32 mg/kg, the dopamine receptor agonist lisuride had no significant effect on either the number of WDS or AD duration (Fig. 3 and Table 1). The inhibition of WDS caused by a dose of 1.0 mg/kg of (+)isofloxythepin or haloperidol was blocked by pretreatment with lisuride.

**Discussion**

In the present study, WDS induced by hippocampal stimulation was markedly and dose-dependently inhibited by the novel neuroleptic drug isofloxythepin, which is a dibenzothiepin derivative. Furthermore, by investigating the effects of both optical isomers of isofloxythepin, the WDS inhibition caused by (-)isofloxythepin was found to be approximately 3 times more potent than that of its (+)-isomer. Metysova and Protiva (14) reported that (-)isofloxythepin is 6–20 times more potent than its (+)-isomer with respect to antagonism of apomorphine-induced chewing and agitation, cataleptic activity in rats and anti-amphetamine activity in mice. From these findings, they concluded that the central anti-dopaminergic activity of isofloxythepin was attributable almost exclusively to the (-)-isomer. In the present experiment, significant differences between both optical isomers of isofloxythepin were also shown to exist in the inhibitory potency of WDS induced by hippocampal stimulation.

Another derivative of dibenzothiepin, zotepine, also caused a significant reduction in the number of WDS induced by hippocampal stimulation. Furthermore, WDS was also significantly suppressed by the injection of other neuroleptic drugs such as haloperidol, chlorpromazine and sulpiride. Isofloxythepin
has a higher affinity for dopamine D₂ receptors than haloperidol (1). It was shown that the affinity of haloperidol for D₂ receptors was almost 3 times greater than the affinity of chlorpromazine, which was similar to the affinity of zotepine (15). The affinity of sulpiride for D₂ receptors was much lower than other neuroleptic drugs (1). Based on these findings, the relative potencies of neuroleptic in the inhibition of WDS were similar to the affinity of these compounds for D₂ receptors, although isofloxythepin was not as potent as predicted from the binding studies. Therefore, it is suggested that the D₂ receptor blocking action is important in the inhibition of WDS induced by hippocampal stimulation. This conjecture is supported by the present results showing that pretreatment with a direct dopaminergic agonist, lisuride (16–18), reversed the WDS suppression caused by isofloxythepin or haloperidol and that a selective D₂ receptor antagonist, sulpiride (19), exerted the inhibitory effect on WDS.

Some neuroleptics (e.g., zotepine) have been reported to possess a relatively potent anti-serotonin action in addition to a dopaminergic blocking action (20). However, since the serotonin antagonist cinanserin, at doses of 3.2–32 mg/kg, i.p., had no significant effect on hippocampal stimulation-induced WDS (10), it seems unlikely that the anti-serotonin action of zotepine accounts for its inhibitory effect on WDS.

In conclusion, it is suggested that isofloxythepin and zotepine, which are structurally novel neuroleptics that are dibenzothiepin derivatives, share an inhibitory action on WDS with other known neuroleptic drugs, and the dopamine D₂ receptor blocking action plays a crucial role in the inhibition of WDS induced by electrical stimulation of the rat hippocampus.

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References


