Effects of Histaminergic Drugs on Muricide Induced by Thiamine Deficiency

Kenji ONODERA and Yasumi OGURA
Department of Pharmacology, Tohoku University School of Dentistry, Sendai 980, Japan
Accepted August 8, 1983

Abstract—Male Wistar rats maintained on a thiamine deficient diet showed mouse-killing aggression (muricide). On the 30th day of experimental feeding, the incidence of this muricide was about 70%. Intracerebroventricular histamine suppressed the muricide induced by thiamine deficiency in a dose-dependent manner. Histamine H1-receptor blocking agents such as diphenhydramine, promethazine and chlorpheniramine also showed muricidal suppression, but astemizole which lacks central effects did not show muricidal suppression. Mianserin and iprindole showed muricidal suppression, but metamizol i.p. did not. On the 20th day of experimental feeding, the incidence of this muricide was 45.5%. Histamine synthesis inhibitors such as brocresine or α-fluoromethylhistidine could not enhance the muricide on non-killer-rats, but really suppressed the thiamine deficient killer-rats. The results of this paper suggested that muricide induced by thiamine deficiency is not mediated by the central histaminergic system, but pharmacologically characterized by antidepressants, antihistamines and histamine synthesis inhibitors.

We have previously found that thiamine deficiency caused a muricidal aggression in male rats (1). We have also reported the characteristics of this muricide and suggested central serotonergic neurons are involved in the inhibition of the muricide aggression in thiamine deficient killer-rats (2, 3). Many investigators have shown that serotonin is predominantly inhibitory on several forms of aggression; the number of killer-rats can be decreased by agents which are believed to activate the central serotonergic system (4–6). Conversely, the number of killer-rats can be increased by brain lesion or drug treatment which depletes the serotonin content in the brain (7, 8). These reports are consistent with previous results that injection of 5-hydroxytryptophan or intracerebroventricular (i.c.v.) serotonin suppressed the muricide, and conversely, parachlorophenylalanine injection facilitated killing aggression (2, 3).

However, certain mechanisms, except for the serotonergic system, are not yet clear in thiamine deficient killer-rats. Recently, Kanof and Greengard suggested that central histamine receptors might be connected with the targets for antidepressants (9). Mianserin and iprindole, which are known as "atypical" antidepressants, do not inhibit the monoamine reuptake system, whereas the inhibition of noradrenaline or serotonin reuptake was believed for many years to be the basis of the clinical action (10, 11). The antihistaminic activity of these antidepressants is well documented in vivo (12, 13) and in clinical investigations (14).

Therefore, we tried to examine the effect of histaminergic drugs including "atypical" antidepressants on the muricide induced by thiamine deficiency, which has been reported as a useful experimental model for evaluating new antidepressants (3, 15). The final purpose of the current study is to examine pharmacologically whether this muricide is mediated by the histaminergic system in thiamine deficient killer-rats.

Materials and Methods

Animals: Male Wistar rats, weighing 60–80 g at the beginning of the experiment, were
obtained from Funabashi Farm Co. Mice weighing 18–22 g were sacrificed for the muricide test. The animals were kept at constant temperature (22±2°C) with a constant relative humidity, and the light cycle was automatically controlled (7:30–19:30 hr). The rats were housed individually in mesh cages (17×25×37 cm) and were provided with a thiamine deficient diet consisting of a basic ration, including 67.6% carbohydrate, 18% lipids and supplemented with vitamins (except thiamine) and minerals. The complete diet was identical to the thiamine deficient diet except that it contained 0.5 mg of thiamine hydrochloride per 100 g of diet (1).

Pharmacological test: One hr before drug administration, the thiamine deficient rats were tested for their tendency to kill (during a 3 min observation period) a mouse which was introduced into the home cages on a certain day of the experimental feedings. When the rat killed the mouse during this period, the rat was labeled as a “killer-rat”, and those which failed to kill were labeled as “non-killer-rats”. The drugs used in this experiment were the antidepressants iprindole HCl (John Wyeth brother Ltd.), mianserin HCl (Nihon Organone and Sankyo Co. Ltd.), and amitriptyline HCl (Nippon Merck Banyu); the antihistamines diphenhydramine HCl (Kowa), chlorpheniramine maleate (Sankyo), promethazine HCl (Yoshitomi), astemizole (Janssen) and metiamide (Smith Kline and French Lab. Ltd.); the histidine decarboxylase inhibitors brocresine dihydrogen phosphate (Ledere) and a-fluoromethyl-histidine 2HCl (Merck Sharp and Dohme). Astemizole and metiamide were suspended in 0.5% tween 80 solution. All other drugs were dissolved in saline solution. The doses were expressed in terms of the salts. All drugs in this pharmacological test were injected intraperitoneally (i.p.).

Intracerebroventricular injection of histamine into killer-rats induced by thiamine deficiency: After 30 days of experimental thiamine deficient feeding, the thiamine deficient killer-rats were given 1 mg/kg thiamine hydrochloride, i.p. These rats were still housed in mesh cages, but were provided the thiamine added complete diet and water ad lib. Twenty days after the injections, the rats still showed muricide aggression. We selected rats weighing 180–220 g for surgery. All animals were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and a cannula, which was a slightly modified metal hypodermic needle (0.5 mm dia.), was implanted into the right ventricle of each rat according to the brain atlas of Pellegrino and Cushman (16). The rats were allowed at least 10 days to recover from the surgical procedure before the test. Histamine 2HCl (Wako) was dissolved in pyrogen-free Ringer’s solution and adjusted to pH 6.0–6.5, and it was given i.c.v. to the animals in a volume of 10 μl per animal with a graduated Hamilton microsyringe. The position of the cannula in the ventricle was verified histologically at the end of the experiment by the injection of thionine blue just prior to sacrificing the animals. Thirty min after i.c.v. histamine, the muricide test was performed for 5 min.

Statistical analysis: Statistical significance among the groups was assessed by the x²-test. The ED50 values were calculated by the method of Litchfield and Wilcoxon (17).

Results

Effect of intracerebroventricular injection of histamine on the muricide induced by thiamine deficiency: Figure 1 shows that muricide induced by thiamine deficiency was dose-dependently suppressed by i.c.v. histamine. Fifty and 100 μg of histamine significantly suppressed this muricide when compared with the Ringer-treated group (P<0.01). The ED50 for muricidal sup-
pression was approx. 26 μg (95% confidence limits: 14.3–45.5 μg).

Effect of histidine decarboxylase inhibitors on the killer-rats and non-killer-rats in thiamine deficiency: On the 20th day of experimental feeding, the incidence of muricide aggression was 45.5% in thiamine deficient rats. The thiamine deficient rats were divided into three groups, that is, α-fluoromethyl-histidine, brocresine and saline treated groups. Each group consisted of 11 thiamine deficient rats (6 non-killer-rats and 5 killer-rats). α-Fluoromethylhistidine at 100 mg/kg i.p. suppressed this muricide in 2 out of 5 killer-rats at 0.5 and 1 hr after the injections. This suppression was also shown in the brocresine-treated group. The suppression by α-fluoromethylhistidine was observed in 3 out of 5 rats from 3 to 24 hr, while the effect of brocresine disappeared at 6 hr. Both brocresine and α-fluoromethylhistidine did not convert non-killer-rats into killer-rats at any of the observation periods (Fig. 2).

Effect of "atypical" antidepressants and antihistamines on the muricide induced by thiamine deficiency: On the 30th day of experimental feedings, the incidence of muricide aggression was about 70%, and none of the rats showed muricide aggression at the beginning of experimental feedings. Table 1 shows the effect of promethazine on

![Fig. 2. Effect of histamine synthesis inhibitors on muricide induced by thiamine deficiency.](image)

Table 1. Effect of antihistamines on muricide induced by thiamine deficiency

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Animals exhibiting suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promethazine</td>
<td></td>
</tr>
<tr>
<td>5 mg/kg i.p.</td>
<td>66.6% (4/6 at 0.5 hr)**</td>
</tr>
<tr>
<td>10 mg/kg i.p.</td>
<td>66.6% (4/6 at 0.5 hr)**</td>
</tr>
<tr>
<td>25 mg/kg i.p.</td>
<td>83.3% (5/6 at 0.5 hr)**</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td></td>
</tr>
<tr>
<td>7 mg/kg i.p.</td>
<td>33.3% (2/6 at 1 hr)</td>
</tr>
<tr>
<td>10 mg/kg i.p.</td>
<td>50.0% (3/6 at 1 hr)*</td>
</tr>
<tr>
<td>25 mg/kg i.p.</td>
<td>100.0% (6/6 at 1 hr)**</td>
</tr>
<tr>
<td>Astemizole</td>
<td></td>
</tr>
<tr>
<td>5 mg/kg i.p.</td>
<td>0% (0/6 at 1 hr)</td>
</tr>
<tr>
<td>10 mg/kg i.p.</td>
<td>0% (0/6 at 1 hr)</td>
</tr>
<tr>
<td>20 mg/kg i.p.</td>
<td>0% (0/6 at 1 hr)</td>
</tr>
<tr>
<td>Metiamide</td>
<td></td>
</tr>
<tr>
<td>5 mg/kg i.p.</td>
<td>0% (0/6 at 0.5, 1, 3, 24 hr).</td>
</tr>
<tr>
<td>10 mg/kg i.p.</td>
<td>0% (0/6 at 0.5, 1, 3, 24 hr).</td>
</tr>
<tr>
<td>20 mg/kg i.p.</td>
<td>0% (0/6 at 0.5, 1, 3, 24 hr).</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0% (0/8 at 0.5, 1, 3, 24 hr).</td>
</tr>
<tr>
<td>0.5% Tween 80 solution</td>
<td>0% (0/8 at 0.5, 1, 3, 24 hr).</td>
</tr>
</tbody>
</table>

( ) indicates the number of rats. The denominator denotes the total number of rats; the numerator denotes the number in which the muricide was suppressed. Statistical differences from the vehicle-treated group (*P<0.05 and **P<0.01).
the muricide induced by thiamine deficiency. The histamine H1-receptor blocking agent promethazine showed dose-dependent muricidal suppression, 30 min after the injections. Five and 10 mg/kg promethazine showed significant muricidal suppression occurring in 4 out of 6 rats, and at 25 mg/kg, in 5 out of 6 rats (P<0.01). Diphenhydramine also produced muricidal suppression dose-dependently, and the ED50 was approx. 8.6 mg/kg at 1 hr (95% confidence limits: 7.5–9.9 mg/kg) as shown in Table 1. Figure 3 shows the effect of chlorpheniramine on the muricide induced by thiamine deficiency in comparison with 5 mg/kg amitriptyline. Five mg/kg chlorpheniramine suppressed this muricide in 3 out of 6 rats, from 0.5 to 1 hr after the injection. On the other hand, amitriptyline, as a reference antidepressant drug, showed muricidal suppression in 5 out of 6 rats at 0.5 hr, and in 3 out of 6 rats at 1 hr, respectively. Ten mg/kg chlorpheniramine produced significant decreases of the number of killer-rats from 0.5 to 3 hr, when compared with the saline-treated group (P<0.01, Table 1). Astemizole had no suppressive effect on the muricide induced by thiamine deficiency from 5 to 20 mg/kg, as shown in Table 1. The histamine H1-receptor blocking agent metiamide did not show any muricidal suppression from 5 to 20 mg/kg at any period (Table 1). Saline and 0.5% tween 80 solution did not show any suppression in these observation periods (Table 1). Figure 4 shows that the muricide was dose-dependently suppressed by mianserin, which is known as an “atypical” antidepressant drug. Ten and 20 mg/kg mianserin produced significant muricidal suppression when compared with the saline-treated group from 0.5 to 3 hr (P<0.01, Table 1 and Fig. 4). The ED50 for muricidal suppression by mianserin at 1 hr was approx. 7.0 mg/kg (95% confidence limits: 3.8–13.0 mg/kg). Figure 5 shows the effect of iprindole on the muricide induced by thiamine deficiency. Fifteen and 20 mg/kg iprindole produced muricidal suppression when compared with the saline-treated group from 0.5 to 3 hr (P<0.05 and P<0.01, Table 1 and Fig. 5). The ED50 for muricidal suppression by iprindole was approx. 11.0 mg/kg at 1 hr (95% confidence limits: 7.6–16.0 mg/kg). Muricidal suppression by both mianserin and iprindole disappeared 24 hr after the injections (Figs. 4 and 5).
Discussion

Since Horovitz et al. found that muricide could be selectively suppressed by various antidepressants, this behavior has been introduced for the pharmacological analysis of this category of drugs (6, 15). However, it has been known that "atypical" antidepressants have little effect on the inhibition of aggression and muricide aggression (18, 19). Yet, both mianserin and iprindole have been shown to be highly effective as antidepressants in several clinical studies (9, 18).

In this experiment, mianserin and iprindole are quite sensitive for suppressing the muricide induced by thiamine deficiency. Additionally, we found previously that muricide induced by thiamine deficiency was quite suppressed by tricyclic antidepressants such as imipramine, clomipramine and desmethylimipramine (3, 15). From these results, the muricide induced by thiamine deficiency is a useful experimental animal model for the evaluation of antidepressants.

Among the various molecular mechanisms of antidepressants, those of mianserin and iprindole were highly effective in inhibiting histamine H2-receptor mediated stimulation of adenylate cyclase activity in guinea-pig homogenates (9, 20, 21). However, metiamide, which is a histamine H2-receptor blocking agent, did not suppress the muricide induced by thiamine deficiency. Generally, histamine H2-receptor blocking agents such as metiamide and cimetidine are hydrophilic and do not cross the blood brain barrier by peripheral injections (22).

On the other hand, histamine H1-receptor blocking agents such as diphenhydramine, promethazine and chlorpheniramine were effective in suppressing the muricide induced by thiamine deficiency. However, astemizole, which is a histamine H1-receptor blocking agent, did not suppress the muricide induced by thiamine deficiency. Astemizole is known to a histamine H1-receptor blocking agent which is free of central effects (23).

These results showed that the suppression of muricide is not due to peripheral histaminergic mechanisms.

It is also known that some antihistamines are positive in tests for antidepressant activity (15, 24, 25). For example, triptenamine and dexchlorpheniramine were effective antagonists of tetrabenazine ptosis in mice, muricide in rats and reserpine hypothermia in mice and rats (24). Our results are also positive and supported the theory that there is a close relationship between antihistaminic activity and antidepressant activity (9, 24). However, in contrast with animal data (15, 24, 25), there is not so much data concerned with the clinical efficacy of antihistamines, except for diphenhydramine (24, 26). Further investigation is needed to clarify this relationship in the clinical efficacy of these drugs.

Histamine synthesis inhibitors such as brocresine (27), and α-fluoromethylhistidine (28) suppressed the muricide induced by thiamine deficiency. It is interesting that α-fluoromethylhistidine, a selective "suicide" inhibitor of histidine decarboxylase, produced long-lasting suppression of this muricide in comparison with brocresine (27–29).

In this experiment, either histamine depletion or histamine receptor blockade was able to interfere with muricide induced by thiamine deficiency. This phenomenon was also reported by Donoso and Broitman who examined the normal copulatory responsiveness of female rats (30).

Another interesting finding was that histamine synthesis inhibitors could not convert non-killer-rats into killer-rats. Nevertheless, i.c.v. histamine suppressed the muricide induced by thiamine deficiency in a dose-dependent manner. If this muricide aggression is mediated by central histamine, the number of killer-rats can be increased by drug treatment which depletes the histamine content in the brain.

However, both activation and inhibition of the central histaminergic system produced the suppression of muricide. These results do not support the inhibitory role of central histamine in thiamine deficient killer-rats, although histamine is believed to exert some of its actions as a neurotransmitter (31, 32).

Finally, whatever the molecular mechanisms underlying the antimuricidal action by histaminergic agents, muricide induced by thiamine deficiency is pharmacologically characterized by antidepressants, antihistamines and histamine synthesis inhibitors in this experiment.
Acknowledgements: The authors are grateful to Dr. M.E. Parsons (Smith Kline and French Lab.), Dr. J. Kollonitsch (Merck Sharp and Dohme), Dr. D.N. Lidge (Ledere), Dr. V.W. Bever (Janssen Pharmaceuticals) Kowa, Yoshitomi, Sankyo, Nihon Organone and Sankyo Co., Ltd., for their generous supplies of histaminergic agents.

References
1 Onodera, K. and Ogura, Y.: Muricide induced by thiamine deficiency in rats. Folia Pharmacol. Japon. 74, 641–648 (1978) (Abs. in English)
19 Kamioka, T. and Sakai, Y.: Behavioral pharmacology of mianserin hydrochloride, a new antidepressant. Folia Pharmacol. Japon. 76, 533–547 (1980) (Abs. in English)
25 Wallach, M.B. and Hedley, L.R.: The effects of


