The Effect of Haloperidol on the Histaminergic Neuron System in the Rat Brain

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Department of Psychiatry, Tohoku University School of Medicine, Sendai 980-77, Department of Pharmacology, Tohoku University School of Dentistry, Sendai 980-77, Department of Medical Physics, School of Allied Health Sciences, Osaka University Faculty of Medicine, Suita 565, and Department of Cellular Pharmacology, Tohoku University School of Medicine, Sendai 980-77

Ito, C., Onodera, K., Yamatodani, A., Yanai, K., Sakurai, E., Sato, M. and Watanabe, T. The Effect of Haloperidol on the Histaminergic Neuron System in the Rat Brain. Tohoku J. Exp. Med., 1997, 183 (4), 285-292 — In this study, the effect of haloperidol on histamine (HA) levels, histidine decarboxylase (HDC) activities and the bindings of [3H]-(R)-α-methylhistamine ([3H]-(R)-α-MeHA) to histamine H1 receptors were investigated in the rat brain. Administration of 10 mg/kg of haloperidol decreased HA levels in the rat striatum and diencephalon, but increased HDC activities in rat striatum and diencephalon, although that of 5 mg/kg did not change them. Meanwhile, haloperidol inhibited the bindings of [3H]-(R)-α-MeHA to H1 receptor sites in the rat striatal membrane with a Ki value of 10.5 ± 0.45 μM. These findings suggest that only a high dose of haloperidol increases HA synthesis and release as a histamine H1 receptor antagonist in the rat brain. ——— haloperidol; histamine level; histidine decarboxylase; histamine H3 receptor; rat brain © 1997 Tohoku University Medical Press

Histamine (HA) is widely distributed in the brain and is recently regarded as a neurotransmitter or neuromodulator (Prell and Green 1986; Schwartz et al. 1991; Wada et al. 1991; Onodera et al. 1994). Cell bodies of HA neurons are localized in the tuberomammillary nucleus in the posterior hypothalamic region, while their varicos fibers are found in almost all regions of the brain (Prell and Green 1986; Schwartz et al. 1991; Wada et al. 1991; Onodera et al. 1994). Synthesis of HA involves transport of L-histidine into the cell and its single step decarboxylation by L-histidine decarboxylase (HDC) (Prell and Green 1986; Schwartz et al. 1991;
Wada et al. 1991; Onodera et al. 1994). HA neuron has histamine H₃ receptors as presynaptic autoreceptors which regulate HA synthesis and release in the mammalian brain (Arrang et al. 1983).

Several lines of evidence were recently obtained that the HA turnover was accelerated in schizophrenia. In clinical study, the mean level of tele-N-methylhistamine in cerebrospinal fluid of chronic schizophrenic patients, an index of histaminergic activity, was higher than in that of controls and correlated positively with severity of schizophrenic symptoms (Prell et al. 1995). Behavioral sensitization, which was induced by repeated treatments with psychostimulants, and phencyclidine model have been established as the animal models of schizophrenia (Bell 1973; Ellinwood et al. 1973; Javitt and Zukin 1991). We recently reported that repeated treatment with methamphetamine increased HA release in the rat striatum at the rechallenge of methamphetamine after drug-free intervals (Ito et al. 1996). Phencyclidine also released HA from cerebral slices by the blockade of a histamine H₃ receptor (Arrang et al. 1988). In the present study, we examined whether haloperidol, a kind of antipsychotic with potent dopamine D₂ blocking action, affected HA level and HDC activity by binding of a histamine H₃ receptor in the rat brain.

**Materials and Methods**

**Animals**

Wistar rats weighing 220-240 g were group-housed (2 rats per cage) with free access to food and water in a room maintained at 22±2°C and 65±5% humidity under a 12 hours light-dark cycle (light on at 6:00 a.m.). This study was conducted in accord with a guide for the care and use of laboratory animals regulated by Tohoku University School of Medicine, and NIH guidelines on animal care.

**Measurement of HA levels**

Rats were sacrificed by decapitation 1 hour after intraperitoneal (i.p.) injection of saline or 5 and 10 mg/kg of haloperidol. Haloperidol was obtained from Dainippon Pharmaceutical Co. (Tokyo). The brains were rapidly removed and dissected on ice into 6 regional parts of the cortex, striatum, diencephalon, midbrain, pons-medulla and cerebellum by the method of Glowinski and Iversen (1966). After the homogenization of a part in 10 volumes (w/v) of 3% perchloric acid containing 5 mM Na₂-EDTA by a Polytron homogenizer (Kinematica, Lucern, Switzerland) at a maximum setting for 10 seconds in an ice bath, the homogenates were centrifuged at 10 000 g for 30 minutes at 4°C. The supernatents were stored at -80°C until HA analyses.

**Measurement of HDC activities**

The parts of the brain taken out as mentioned above were homogenized in 10 volumes (w/v) of HDC solution (100 mM potassium phosphate buffer, pH 6.8,
0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1% polyethylene glycol and 100 μg/ml phenylmethanesulfonylfluoride) in a Polytron homogenizer (Kinematica) at a maximum setting for 10 seconds in an ice bath. The homogenates were centrifuged at 10,000 g for 30 minutes and the supernatants were dialyzed against HDC solution overnight. The HDC reaction was started at 37°C by 0.5 mM L-histidine (final concentration) in a total volume of 1.0 ml. Three hours later, the reaction was stopped by adding 0.03 ml of 60% perchloric acid. The mixtures were briefly centrifuged, and the supernatants were stored at −80°C until HA analyses. Proteins were determined with bovine serum albumin as standard by the method of Lowry et al. (1951).

**HA analyses**

HA was measured by a sensitive HPLC-fluorometric method as described by Yamatodani et al. (1985). Briefly, HA was separated on a cation exchanger, TSK gel SP2SW (particle size 5 μm; Tosoh, Tokyo) eluted with 0.25 M KH₂PO₄ at a flow rate of 0.6 ml/min using a constant flow pump (Model CCPM; Tosoh). HA eluate was derivatized using an on-line automated Shore's o-phthalaldehyde method (1951), and the fluorescence intensity was measured at 450 nm with excitation at 360 nm in a spectrofluorometer equipped with a flow cell (Model C-R3A; Shimadzu, Kyoto) and a chromatographic data processor (Model C-R3A; Shimadzu).

[^3H]-{(R)-α-methylhistamine binding assays}

Membrane fractions were obtained from the rat brains, and[^3H]-{(R)-α-methylhistamine ([^3H]-(R)-α-MeHA) binding was measured as described by Arrang et al. (1983). Briefly, the striatum was homogenized in a Polytron (setting 8, 20 seconds; Kinematica) in 40 volumes (w/v) of ice-cold 50 mM Na-K phosphate buffer, pH 7.4. In subcellular distribution studies, the homogenates were centrifuged twice at 1000 g at 4°C for 10 minutes to remove the nuclear fraction and cell debris. The supernatants were combined and then centrifuged at 50,000 g for 20 minutes to obtain the membrane fraction. The binding assay of[^3H]-(R)-α-MeHA was performed by a slight modification of method by Arrang et al. (1983).[^3H]-(R)-α-MeHA (39 mCi/μmol) was obtained from Amersham Co., Ltd. (Buckinghamshire, England). Briefly, for the inhibition analysis, 0.4 ml aliquots of the suspensions (10 mg weight tissue) were incubated for 60 minutes at 25°C with 1.0 nM[^3H]-(R)-α-MeHA and haloperidol of 0.5 nM to 5 mM in a final volume of 0.5 ml. The specific binding was defined as that inhibited by 10 μM thioperamide. Thioperamide was obtained from Research Biochemicals International (Natick, MA, USA). The reactions were terminated by addition of 5 ml of the ice-cold buffer and rapid filtration on a glass fiber filter (GF/B) precoated with 0.3% polyethyleneimine. The filters were washed with 5 ml volumes of cold buffer 3 times, and the radioactivities trapped on the filters were
counted in 10 ml of aquasol 2 (Amersham Co., Ltd.) with a scintillation counter.

Data analyses

The data of binding assay were analyzed to determine the IC₅₀ values for haloperidol by fitting the data with the iterative computer least-squares method derived from Parker and Waud (1971). The apparent dissociation constant (Ki value) for haloperidol was calculated from its IC₅₀ value, assuming a competitive antagonism and neglecting the influence of endogenous HA, according to the equation of Cheng and Prusoff (1973): Ki = IC₅₀/(1 + L/Kd), where L represents the [³H]-(R)-α-MeHA concentration (1.0 nM) and Kd the dissociation constant of [³H]-(R)-α-MeHA (0.5 nM).

Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan's test. In all cases, p values less than 0.05 were considered statistically significant.

Results

Effects of haloperidol on HA levels

Administration of 10 mg/kg of haloperidol (i.p.) decreased HA levels in the striatum and diencephalon, although that of 5 mg/kg did not change them (Table 1). There were no significant changes in HA levels of other regions after administration of haloperidol (i.p.) (Table 1).

Effects of haloperidol on HDC activities

Administration of 10 mg/kg of haloperidol (i.p.) increased HDC activities in the striatum and diencephalon, although that of 5 mg/kg did not change them (Table 2). There were no significant changes in HDC activities of other regions after administration of haloperidol (i.p.) (Table 2).

| Table 1. The effect of haloperidol on histamine level in the rat brain |
|------------------------|--------|----------------|--------|--------|--------|
|                        | Cortex | Striatum       | Diencephalon | Midbrain | Pons-medulla | Cerebellum |
|------------------------|--------|----------------|--------|--------|--------|
| Saline                 | 227.0  | 241.2          | 1728.8 | 286.4  | 196.3  | 28.8       |
| ± 31.8                 | ± 13.0 | ± 238.2        | ± 67.6 | ± 90.0 | ± 8.8  |
| Haloperidol (5 mg/kg)  | 264.0  | 257.3          | 1335.1 | 206.0  | 199.8  | 34.5       |
| ± 25.3                 | ± 24.4 | ± 190.8        | ± 10.7 | ± 42.3 | ± 10.8 |
| Haloperidol (10 mg/kg) | 260.5  | 173.6          | 829.8  | 246.4  | 213.1  | 34.0       |
| ± 91.8                 | ± 11.5**| ± 50.5*        | ± 40.1 | ± 47.8 | ± 10.4 |

Rats were sacrificed 1 hour after administrations of saline (1 ml/kg, i.p.) and haloperidol (5 and 10 mg/kg, i.p.). Each value represents the mean ± S.E.M. (pmol/g wet tissue) of 6 rats. Statistical analysis was performed by means of one way ANOVA followed by Duncan's test (*p < 0.05, **p < 0.01).
Effects of haloperidol on the binding of $[^3H]-(R)-\alpha$-MeHA

Haloperidol inhibited the binding of $[^3H]-(R)-\alpha$-MeHA to membrane fractions of the striatum with an IC$_{50}$ value of $31.6 \pm 1.36 \mu M$ and a pseudo-Hill coefficient of $0.909 \pm 0.105$ in the striatum (Fig. 1). A calculated Ki value of $10.5 \pm 0.45 \mu M$ was found for haloperidol.

Table 2. The effect of haloperidol on histidine decarboxylase activity in the rat brain

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Striatum</th>
<th>Diencephalon</th>
<th>Midbrain</th>
<th>Pons-medulla</th>
<th>Cerebellum</th>
</tr>
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<td>119.5</td>
<td>380.4</td>
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<td>± 22.2</td>
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<td>± 15.5</td>
<td>± 14.9</td>
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<tr>
<td>Haloperidol</td>
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<td>131.4</td>
<td>404.2</td>
<td>179.6</td>
<td>48.1</td>
<td>10.9</td>
</tr>
<tr>
<td>(5 mg/kg)</td>
<td>± 11.3</td>
<td>± 8.5</td>
<td>± 63.4</td>
<td>± 19.8</td>
<td>± 8.0</td>
<td>± 1.2</td>
</tr>
<tr>
<td>Haloperidol</td>
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<td>173.6</td>
<td>513.1</td>
<td>190.2</td>
<td>59.6</td>
<td>7.3</td>
</tr>
<tr>
<td>(10 mg/kg)</td>
<td>± 28.3</td>
<td>± 11.5**</td>
<td>± 47.1*</td>
<td>± 20.9</td>
<td>± 5.8</td>
<td>± 1.3</td>
</tr>
</tbody>
</table>

Rats were sacrificed 1 hour after administrations of saline (1 ml/kg, i.p.) and haloperidol (5 and 10 mg/kg, i.p.). Each value represents the mean±s.e.m. (fmol/min/mg protein) of 6 rats. Statistical analysis was performed by means of one way ANOVA followed by Duncan’s test (*p<0.05, **p<0.01).

Fig. 1. The inhibition by haloperidol of the $[^3H]-(R)-\alpha$-MeHA binding to the striatal membrane in rats. Membranes (10 mg wet tissue) were incubated for 60 minutes at 25°C with 1.5 nM of $[^3H]-(R)-\alpha$-MeHA and increasing concentrations of haloperidol. The specific binding was defined as that inhibited by 10 $\mu M$ thiopropamide. Results are expressed as percentages of this value, each point representing the results from 6 different experiments with triplicated determination.


DISCUSSION

In the present study, we first found that a low dose of a dopamine D2 antagonist haloperidol which is clinically effective to antipsychotic action affected neither HA levels nor HDC activities, while we recently reported that methamphetamine increased HA release mediated by dopamine D2 receptors (Ito et al. 1996). We also found that haloperidol was a histamine H3 receptor antagonist of low potency. It is well known that histamine H3 receptor is presynaptic autoreceptors which regulate HA synthesis and release in the brain (Arrang et al. 1983). The Ki value of haloperidol in this study is similar to that obtained with [3H] N-α-MeHA or [125I]-iodophenpropit as a tracer in the rat brain (Kathmann et al. 1994; Rodrigues et al. 1995). These findings suggest that the effective dose to antipsychotic of haloperidol dose not act to the HA neuron system, because haloperidol is not only dopamine D2 antagonist but also a histamine H3 receptor antagonist of low potency.

Administration of a high dose of haloperidol, however, decreased HA levels in the rat striatum and diencephalon. HDC activities in the rat striatum and diencephalon also increased by administration of a high dose of haloperidol. These findings suggest that a high dose of haloperidol increases HA turnover, because HDC activity is a sign of HA turnover. These findings are consistent with a high dose of haloperidol-induced increase of HA turnover in the whole brain of mice (Yagi et al. 1995). A high dose of perphenazine also increased HA turnover and HDC activity in the whole brain of mice and rats (Chopra and Dandiya 1975; Malec and Langwinski 1983; Yagi et al. 1995). Sunderland and Cohen (1986) reported that when 1 mg/kg of haloperidol (i.p.) was administrated, its concentration in the rat striatum reached about 1 μM. Therefore, the effect of 10 mg/kg of haloperidol on HA level and HDC activity may result from the antagonism of haloperidol to H3 receptors, because the Ki value is 10 μM.

A high dose of haloperidol is occasionally administrated to the patient of chronic schizophrenia (Clark and Holden 1987). The increase of HA turnover was reported to be related to the brain dysfunction of chronic schizophrenia (Prell et al. 1995). From these findings, a high dose of haloperidol must not be administrated, because it may worsen the symptom of chronic schizophrenia. It was also reported that treatments with the agents that activate the HA neuron system enhanced antipsychotic-induced catalepsy, whereas those that inhibit the HA neuron system attenuated it in mice and rats (McGeer et al. 1971; Chopra and Dandiya 1975; Pilc et al. 1982; Malec and Langwinski 1983). Moreover, several reports showed that the loading of L-histidine induced cataleptic-like syndrome or bizarre behavior in rats (Maslinski et al. 1973; Yagi et al. 1995), and that the intracerebroventricular administration of HA induced catalepsy in rats (Nowak and Polic 1975; Glick and Crane 1978). These findings suggest that the activation of the HA neuron system induced by haloperidol may be related to the
haloperidol-induced side effect.

In conclusion, in the present study, a high dose of haloperidol increased HA turnover and HDC activity as a histamine H₃ receptor antagonist in the rat brain.

Acknowledgments

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References


