SEX DIFFERENCE IN THE DEVELOPMENT OF HYPERSENSITIVITY OR TOLERANCE TO HALOPERIDOL IN THE RAT

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Abstract—Sex difference in the cataleptic response to continuous or intermittent administration of haloperidol (1 mg/kg, i.p.) was examined in the rat. Weekly administration of haloperidol induced a hypersensitivity to haloperidol itself to a greater extent in adult female rats as compared to adult males. Five daily injections of haloperidol induced a marked tolerance to haloperidol in adult female rats but not in males. Ovariectomy in adult rats failed to alter the development of hypersensitivity or tolerance to haloperidol. Orchiectomy in adult rats resulted in the development of a hypersensitivity to haloperidol during the weekly administration and a tolerance during daily injection of haloperidol. In immature female rats, weekly administration begun at 3 weeks of age induced a marked increase in the intensity of haloperidol-induced catalepsy at 7 weeks of age. Daily injection of haloperidol in 3-week-old rats did not show any significant sex difference. These findings suggest that exposure to sex hormones, probably during the time of puberty onset, results in a modification of the activity of dopaminergic and/or related neurons responsible for cataleptic behavior to female and male types. Female sex hormones appear to induce a persistent modification of the dopaminergic system at a certain critical period during the maturation.

Continuous administration of haloperidol or phenothiazine neuroleptics in the rats is known to induce behavioral supersensitivity to apomorphine, a dopaminergic agonist (1–3). On the other hand, repeated administration of haloperidol at various intervals has been found in the rat to develop a hypersensitive response to haloperidol when the discriminated conditioned avoidance was tested (4). In these experiments, male animals were exclusively employed, and behavioral experiments in which female animals were used are not common. Currently, Flemenbaum (5) compared the effect of chronic amphetamine treatment in both sexes of rats and found behavioral supersensitivity in response to apomorphine in male rats but not in female animals. Koller et al. (6) have reported that behavioral supersensitivity to apomorphine following chronic treatment with haloperidol can be induced in ovariectomized guinea pigs.

We have found that weekly administration of haloperidol in young adult rats induces hypersensitivity to haloperidol itself to a greater extent in females as compared with males when the effect of haloperidol was assessed on the basis of cataleptic behavior (7). The present experiment was undertaken to further investigate the sex difference in the
development of hypersensitive state in haloperidol-induced catalepsy. We found that daily administration of haloperidol induces a marked tolerance to haloperidol in female rats but not in males and that ovariectomy in adult rats did not affect the development of either hypersensitivity or tolerance to haloperidol.

MATERIALS AND METHODS

Immature or adult male and female rats of the Wistar-Imamichi strain were obtained from the Institute for Animal Reproduction (Saitama). Experimental groups consisting of male and female rats were as follows: 1) intact adult, 2) gonadectomized adult, and 3) intact immature rats. Animals of these 3 groups received weekly or daily (i.p.) injection with 1 mg/kg haloperidol and were tested for cataleptic response. Haloperidol solution was prepared by diluting Serenate® (Serenace Injection, Dainippon Pharmaceutical Co., Ltd.) with distilled water. In all experiments, haloperidol was injected between 9:30 and 10:00 a.m. in order to avoid the circadian fluctuation (7) in the effect of haloperidol. Weekly administration of haloperidol in adult rats was begun at 9 weeks of age (males weighing 234±4.5 g, females weighing 198±2.5 g) and daily injection at 12-13 weeks of age (males weighing 400±20.8 g, females 274±9.5 g). In the experiment of weekly administration of haloperidol, the interval between the 1st and 2nd drug injection was 2 weeks and the next 2 injections were given at one week interval as applied in the previous experiment (8) in which the induction of hypersensitivity was found. In order to confirm the previous results, the same time schedule was used. Orchiectomy or ovariectomy in adult rats was performed under ether anesthesia. Control rats were sham operated. Gonadectomized rats were tested for the cataleptic effect of haloperidol 1 week or 3 months after the operation. Haloperidol-induced catalepsy was determined with a bar method (9). The forepaws of adult rats were placed on a horizontal bar with a diameter of 8 mm and located at 8 cm above the surface of a table. For the male rats older than 11 weeks of age, the bar was placed 10 cm high. For immature rats, 3 to 6 weeks of age, the bar was 5 mm in its diameter and placed 4 to 5 cm high. The intensity of catalepsy was assessed as the time during which rats maintained the abnormally imposed position. Once the rats put their forepaws on the table, the paws were placed again on the bar and when they removed the paws from the bar within 5 min, time counting was discontinued. The rats were fed with a stock diet and water ad libitum. The animals were kept in a temperature-conditioned room (22±3°C) with a lighting schedule of 14 hr light (6:00-20:00) and 10 hr darkness. Statistical analysis was carried out using the Student's t-test.

RESULTS

Weekly administration of haloperidol: A single injection of haloperidol (1 mg/kg) to intact adult rats induced cataleptic behavior for approx. 15 min, and there was no significant sex difference in the intensity of catalepsy (Fig. 1). When haloperidol in the same dose was injected 2 weeks after the 1st injection, the duration of the catalepsy showed a slight increase in both male and female rats. The intensity of catalepsy increased markedly at the 3rd treatment with haloperidol; the magnitude of the increment in the intensity of catalepsy in female rats was greater than in males. At the 4th injection, the duration of haloperidol-induced catalepsy in male rats remained unchanged from the value of the 3rd session, whereas female rats showed a further increase in the duration of catalepsy, leading to a marked sex difference in the intensity of catalepsy.
Weekly administration of haloperidol to orchiectomized adult rats induced a marked increase in the duration of catalepsy at the 3rd session afterward (Fig. 2). The intensity of catalepsy in orchiectomized rats was much greater than in ovariectomized adult females. The changing pattern in the cataleptic response in ovariectomized rats was similar to that in intact females shown in Fig. 1, though the duration of catalepsy at the 1st and 2nd session was longer than that in intact rats in Fig. 1.

In immature male rats, the intensity of haloperidol-induced catalepsy tended to decrease from the initial value at the 2nd session afterward (Fig. 3). In contrast, the intensity of haloperidol-induced catalepsy in immature female rats varied depending on the age tested (Fig. 3). Intensity of catalepsy in these rats tended to increase at the 3rd session (6 weeks of age), and a marked enhancement in the cataleptic response was noted at the 4th session (7 weeks of age).

Daily administration of haloperidol: Daily injection of haloperidol at 24-hr intervals to adult rats induced a marked reduction in the intensity of catalepsy in female rats but not in males (Fig. 4). Intensity of the catalepsy in male rats showed a slight increase at the 2nd session and then a decrease toward the level below the initial value at the 3rd and 4th sessions. The value at the 5th session tended to increase over the initial level, though it was not statistically significant.

Ovariectomy in adult rats failed to alter the intensity of haloperidol-induced catalepsy.
when tested 1 week (Fig. 5) or 3 months (Fig. 6) after the operation; a marked and progressive decline in the intensity of the catalepsy similar to that shown in sham-operated controls was observed. In contrast, orchiectomized adult rats showed a slight increase in the duration of catalepsy at the 2nd session and a decrease thereafter.

**Fig. 3.** Cataleptic response to the weekly administration (with the interval as in the legend of Fig. 1) of haloperidol (1 mg/kg, i.p.) in intact immature (3-week-old) rats. Vertical bars represent the mean±S.E.M. The number in the column indicates the number of rats tested. **, P<0.01 vs. the initial value.

**Fig. 4.** Cataleptic response to the daily injection of haloperidol (1 mg/kg, i.p.) in intact adult (13-week-old) rats. Vertical bars represent the mean±S.E.M. The number in the column indicates the number of rats tested. ***, P<0.01 vs. the initial value.

**Fig. 5.** Cataleptic response to the daily injection of haloperidol (1 mg/kg, i.p.) in the gonadectomized adult (12-week-old) rats. They were tested one week after the operation. Vertical bars represent the mean±S.E.M. The number in the column indicates the number of rats tested. *, P<0.05; **, P<0.01 vs. the initial value.

**Fig. 6.** Cataleptic response to the daily administration of haloperidol (1 mg/kg, i.p.) in gonadectomized adult rats 3 months after the operation. Vertical bars represent the mean ±S.E.M. The number in the column indicates the number of rats tested. *, P<0.05; **, P<0.01 vs. the initial value.
reaching a value at the 5th session that was lower than that at the 1st session when tested 1 week after the operation, though there was no significant difference between the sham-operated control and orchiectomized rats. At 3 months after the operation, the changing pattern of the cataleptic response to daily administration of haloperidol in the sham-operated control and orchiectomized rats was similar to that observed 1 week after the operation, though a significant increase in the intensity at the 2nd session and a significant decrease at the 4th and 5th session from the initial value were noted in orchiectomized rats. There was a significant difference in the intensity of catalepsy between sham-operated control and orchiectomized rats at each session except the 2nd session. This was mainly due to the over-all increase in the response to haloperidol in sham-operated rats as compared to that shown 1 week after the operation.

In immature rats (Fig. 7), the duration of catalepsy in male rats tended to be longer than in females at the 1st to 3rd injections of haloperidol. The duration of catalepsy in female rats tended to increase at the 2nd to 4th injections of haloperidol and it returned to the initial level at the 5th injection. Immature female rats did not show any marked tolerance to haloperidol after 5 daily injections.

**DISCUSSION**

The present results clearly indicate that intermittent, weekly administration of haloperidol in the adult female rat rapidly induces hypersensitivity to haloperidol itself, whereas daily administration of haloperidol in the adult female rat very rapidly induces a marked tolerance to haloperidol.

Little is known about the effects of sex hormones on the neurotransmitter functions outside the hypothalamus. Recently, it has been reported that estrogen can modulate the behavioral (5, 6) and biochemical (10–12) activity associated with dopaminergic mechanisms. Ovariectomy in the guinea pig reduces their behavioral response to apomorphine and d-amphetamine (10). Estrogen treatment in male rats has been demonstrated to increase the number of striatal dopamine receptors (11, 12). In the present experiment, unexpectedly, ovariectomy in adult rats failed to alter the development of either hypersensitivity or tolerance in the cataleptic response following repeated administration of haloperidol. In immature rats, no significant sex difference in the response to haloperidol was observed during the daily administration of haloperidol. The final injection of haloperidol was at 25 days of age, prepubertal age in the rat. On the other hand, a marked increase in the duration of catalepsy appeared at 7 weeks of age in female rats during the intermittent administration of haloperidol. The vaginal opening in the female rat begins around 5 weeks of age. The cyclic fluctuation in the serum

![Fig. 7. Cataleptic response to the daily administration of haloperidol (1 mg/kg, i.p.) in intact immature (3-week-old) rats. Vertical bars represent the mean ± S.E.M. The number in the column indicates the number of rats tested. **: P<0.01 vs. the initial value.](image)
estrogen level dependent on the sexual cycle can be detected at 7 weeks of age in rats (Fujii, unpublished data). Therefore, these rats could be exposed to a certain critical level of female sex hormones for manifestation of the female pattern in cataleptic behavior. A critical period of exposure to female sex hormones may be around the time of puberty onset. The present results also indicate that female sex hormones appear to manifest a persistent effect on the functional development of dopaminergic system responsible for cataleptic behavior. Becker and Ramirez (13) examined a sex difference in amphetamine-stimulated dopamine release from the striatum in vitro and suggested that the presence of ovaries during maturation, possibly the time associated with puberty, is critical for the development of gonadal steroid modulation of striatal dopamine activity.

The changing pattern of the intensity of catalepsy in sham-operated male rats (Fig. 5) was different from that in intact male rats (Fig. 4), though it was very similar in female rats between these two groups. In other experiments in our laboratory, however, the time course of changes in cataleptic response to daily administration of haloperidol in adult male rats has been found to be similar to that shown in Fig. 4. The cause of this difference between the two groups of male rats in the present experiment is not known. A possibility of long-lasting effects of operation and/or ether anesthesia on the dopaminergic activity remains to be elucidated. Intensity of catalepsy in sham-operated male rats increased when tested 3 months after the operation as compared with that shown one week after the operation. This could be due to the hypersensitivity developed by haloperidol given at the test carried out one week after the operation. It has been reported that a single dose of haloperidol (14) or amphetamine (15) can cause long-lasting changes in certain brain neurons. Moreover, continuous administration for one year of trifluoperazine or thioridazine, dopaminergic antagonists, to rats exhibits long-lasting alterations in the brain as long as for 6 months following their withdrawal (16).

In adult male rats, the development of hypersensitivity to haloperidol was less marked, but orchietomy resulted in a marked prolongation of the duration of haloperidol-induced catalepsy during the intermittent administration of haloperidol. In addition, orchietomized adult rats developed tolerance during the daily administration of haloperidol, though it was demonstrated only 3 months after removal of the gonads. These findings may suggest that testicular androgens play a primary role in manifestation of the male pattern shown in adult rats in the cataleptic response to continuous or intermittent administration of haloperidol. However, modification of the dopaminergic activity by androgens appears to be irreversible. It is of interest that the alteration in cataleptic response to daily administration of haloperidol in the male rats was observed only 3 months after orchietomy. Slow onset of the modification by estrogen of the number of dopamine receptors in male rats has been reported (10). Regardless of the mechanism involved, these evidence may suggest that the modification of the cataleptic behavior is mediated indirectly by sex steroids.

Not only dopaminergic (9) but also noradrenergic (17, 18) or cholinergic (19) neurons in the striatum and mesolimbic system have been suggested to be involved in the manifestation of neuroleptic catalepsy in the rat. Long-term administration of haloperidol or other dopaminergic antagonists to the rat have been observed to result in an increase in the binding site for dopamine agonist in the striatum and mesolimbic areas (20–22) or in an increase in the activity of
striatal adenylate cyclase (16). The mechanism of the development of the sex difference in the activity of the dopaminergic system and/or related areas responsible for cataleptic behavior, together with the mechanism of the development of hypersensitivity or tolerance to haloperidol must await further experiments.

REFERENCES

1) Tarsy, D. and Baldessarini, R.J.: Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. Neuropharmacology 13, 927–940 (1974)
2) Waddington, J.L. and Gamble, S.J.: Neuroleptic treatment for a substantial proportion of adult life: Behavioural sequelae of 9 months haloperidol administration. Europ. J. Pharmacol. 67, 363–369 (1980)
3) Racagni, F., Bruno, A., Bugatti, A., Parenti, M., Apud, J.A., Santini, V., Carenzi, A., Groppetti, A. and Cattabeni, F.: Behavioral and biochemical correlates after haloperidol and clozapine long-term treatment. In Advances in Biochemical Psychopharmacology, Edited by Cattabeni, F., Racagni, G., Sponzo, P.F. and Costa, E., Vol. 24, p. 45–51, Raven Press, New York (1980)
4) Hayashi, T. and Tadokoro, S.: Influence of the environment on the effect of repeated administration of neuroleptics. Biol. Sci. 32, 75–82 (1980) (in Japanese)
5) Flemenbaum, A.: Failure of apomorphine to induce dopamine receptor hypersensitivity. Psychopharmacology 62, 175–179 (1979)
6) Koller, W.C., Weiner, W.J., Klawans, H.L. and Nausieda, P.A.: Influence of female sex hormones on neuroleptic-induced behavioral supersensitivity. Neuropharmacology 19, 387–391 (1980)
7) Nagayama, H., Takagi, A., Sakurai, Y., Yoshimoto, S., Nishiwaki, K. and Takahashi, R.: Studies on circadian susceptibility rhythm to haloperidol. Folia pharmacol. Japon. 74, 951–957 (1978) (Abs. in English)
8) Ikeda, H.: Pharmacological studies on the functional development of the central nervous system in the first generation rats born to phenytoin-treated mothers. Folia pharmacol. Japon. 79, 65–78 (1982) (Abs. in English)
9) Sanberg, P.R.: Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 284, 472–473 (1980)
10) Nausieda, P.A., Koller, W.C., Weiner, W.J. and Klawans, H.L.: Modification of postsynaptic dopaminergic sensitivity by female sex hormones. Life Sci. 25, 521–526 (1979)
11) Hruska, R.E. and Silbergeld, E.K.: Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model. Science 208, 1466–1468 (1980)
12) Hruska, R.E., Ludmer, L.M. and Silbergeld, E.K.: Characterization of the striatal dopamine receptor supersensitivity produced by estrogen treatment of male rats. Neuropharmacology 19, 923–926 (1980)
13) Becker, J.B. and Ramirez, V.D.: Experimental studies on the development of sex differences in the release of dopamine from striatal tissue fragments in vitro. Neuroendocrinology 32, 168–173 (1981)
14) Bannet, J., Belmaker, R.H. and Ebstein, R.P.: The effect of drug holidays in an animal model of tardive dyskinesia. Psychopharmacology 69, 223–224 (1980)
15) Fuller, R.W. and Henrick-Luecke, S.: Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. Science 209, 305–307 (1980)
16) Close, A., Theodorou, A., Jenner, P. and Marsden, C.D.: Cerebral dopamine function in rats following withdrawal from one year continuous neuroleptic administration. Europ. J. Pharmacol. 63, 145–157 (1980)
17) Al-Shabibi, U.M.H. and Doggett, N.S.: On the central noradrenergic mechanism involved in haloperidol-induced catalepsy in the rat. J. Pharm. Pharmacol. 30, 529–531 (1978)
18) Tsukamoto, T., Asakura, M. and Hasegawa, K.: Effects of acute and chronic treatment of desipramine on α-noradrenergic function. Japan. J. Neuropsychopharmacol. 3, 27–33 (1981) (in Japanese)
19) Costall, B. and Naylor, R.J.: The importance of the ascending dopaminergic systems to the extrapyramidal and mesolimbic brain areas for the cataleptic action of the neuroleptic and cholinergic agents. Neuropharmacology 13, 353–364 (1974)
20) Ebstein, R.P., Pickholz, D. and Belmaker, R.H.: Dopamine receptor changes after long-term haloperidol treatment in rats. J. Pharm. Pharmacol. 31, 558–559 (1979)
21) Bannon, M.J., Bunney, E.B., Zigun, J.R., Skirboll, L.R. and Roth, R.H.: Presynaptic dopamine receptors: Insensitivity to kainic acid and the development of supersensitivity following chronic haloperidol. Naunyn-Schmiedeberg’s Arch. Pharmacol. 312, 161–165 (1980)
22) Theodorou, A., Gommeren, W., Clow, A., Leysen, J., Jenner, P. and Marsden, C.D.: Chronic neuroleptic treatment specifically alters the number of dopamine receptors in rat brain. Life Sci. 28, 1621–1627 (1981)