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Repeated Low Dose Apomorphine Induced Subsensitivity of Presynaptic Dopamine Receptors

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MASUDA, Y., S. MURAI, H. SAITO, I. KOHORI AND T. ITOH. *Repeated low dose apomorphine induced subsensitivity of presynaptic dopamine receptors.* PHARMACOL BIOCHEM BEHAV **28**(1) 35–37, 1987.—The influence of repeated administration of a low dose of apomorphine on presynaptic dopamine (DA) receptors was examined. (1) Male ddY mice were given a low dose of apomorphine (0.2 mg/kg) for 7 days. Following 2 drug-free days they were given apomorphine (0.2 mg/kg, IP), after which cage climbing behavior and the level of 3-methoxytyramine (3MT) in striatum was measured. Mice became tolerant to apomorphine's activity-depressing effect and 3MT-decreasing effect. (2) Mice were given haloperidol (1 mg/kg) for 7 days. Then after 2 drug-free days they were given 0.2 or 1.0 mg/kg of apomorphine, after which cage climbing behavior was measured. One mg/kg of apomorphine significantly increased cage climbing behavior, indicating that supersensitivity of postsynaptic DA receptors was induced by haloperidol, but the activity-depressing effect of 0.2 mg/kg of apomorphine was not modified. The results suggest that subsensitivity of presynaptic DA receptors participates in the tolerance to the activity-depressing effect of a low dose of apomorphine.

Low dose apomorphine Subsensitivity Climbing behavior 3-Methoxytyramine Haloperidol Mouse Dopamine receptor

APOMORPHINE has a biphasic effect on the behavior of small laboratory animals. That is to say, with a high dose a marked behavioral stereotypy is produced [6], probably as the result of direct activation of postsynaptic dopamine (DA) receptors [4], and with a low dose it preferentially stimulates presynaptic DA receptor sites [8], reduces DA release from dopaminergic nerve terminals and induces hypomotility. Many studies have shown that presynaptic DA receptors in nigro-striatal projection correlate with the effect of apomorphine to decrease DA function [9, 14, 17]. Recently, several studies [1, 5, 11] have suggested that the effect on behavior of a low dose of apomorphine is diminished by repeated or continuous administration. As to the mechanism of this phenomenon, some investigators [11] have suggested that behavioral tolerance of a low dose of apomorphine results from disuse supersensitivity of postsynaptic DA receptors caused by chronic reduction of DA release, whereas others [1,5] have suggested that it results from a down regulation of presynaptic DA receptors caused by repeated stimulation to the receptor. The present study was undertaken to examine whether the change of sensitivity to presynaptic DA receptors in mice striatum participates in behavioral tolerance of a low dose of apomorphine.

METHOD

Subject

Male ddY mice (initially weighing 22–24 g) were housed, 8 per cage, with food and water ad libitum, and maintained under a 12-hr light dark cycle at 22–24°C ambient temperature, throughout the experimental period. The mice were divided into ten groups. The 1st and 2nd group was each given a low dose of apomorphine (0.2 mg/kg). The 3rd and 4th group was each given a saline solution (0.9% NaCl). Both were administered for 7 days, twice a day at 9 a.m. (intraperitoneal injection; IP) and at 6 p.m. (subcutaneous injection; SC), except on the 7th day, when it was administered only once, at 9 a.m. (IP). Then after 2 drug-free days, apomorphine (0.2 mg/kg, IP) was administered to all four groups, after which cage climbing behavior of the 1st and 3rd groups was measured. 3-Methoxytyramine (3MT) in striatum was also measured using the 2nd and 4th groups. The 5th and 6th groups served as non-treated control groups.

The 7th and 8th group was each given haloperidol (1 mg/kg, IP, once a day for 7 days) to introduce a supersensitivity of postsynaptic DA receptors [2,16]. The 9th and 10th group was each given saline (IP, once a day for 7 days). Then after 2 drug free-days, the 7th and 9th group was each given apomorphine (0.2 mg/kg, IP) and the 8th and 10th group was each given a high dose of apomorphine (1 mg/kg, IP), after which cage climbing behavior was measured.

Behavioral Testing

Cage climbing behavior was measured using the method of Protais *et al.* [15] to assess the stimulation of striatal DA

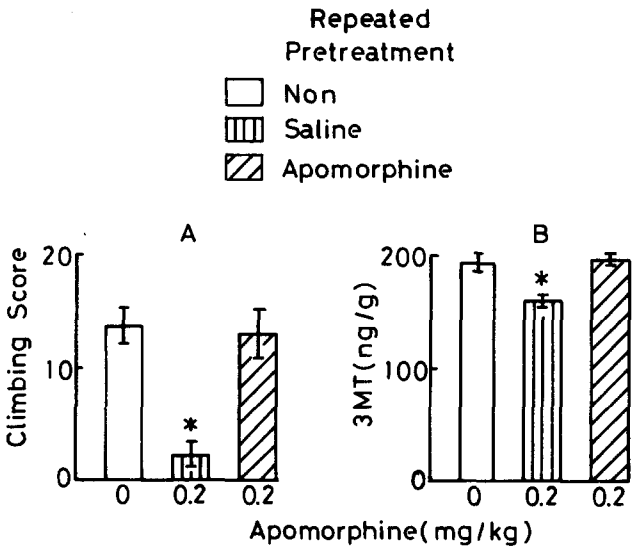

FIG. 1. Changes in the climbing behavior (A) and in the level of 3MT (B) resulting from apomorphine after having repeated administration of apomorphine or saline. Apomorphine (0.2 mg/kg) or saline solution injected daily for 7 days (for further explanation see the Method section). Then after 2 drug-free days, apomorphine (0.2 mg/kg, IP) was injected and the climbing behavior and the level of 3MT was measured. The control group was injected only with the saline solution on the day of measurement. Values represent the mean±SEM (N=8 for A, N=6 for B). ANOVA gave a significant difference between the groups: $F(2,21)=11.898$, $p<0.005$ for A; $F(2,15)=7.77$, $p<0.005$ for B. $*p<0.05$ (compared with control).

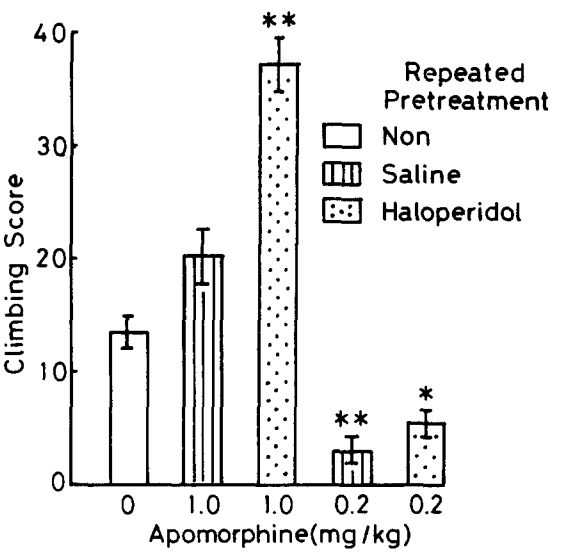

FIG. 2. Changes in the climbing behavior resulting from apomorphine (0.2 and 1.0 mg/kg, IP) after having repeated administration of haloperidol (1 mg/kg). Mice were injected daily with haloperidol or saline for 7 days. Then after 2 drug-free days, apomorphine was injected and the climbing behavior was measured. The control group was injected only with saline solution on the day of measurement. Values represent mean±SEM (N=8). ANOVA gave a significant difference: $F(4,35)=54.077$, $p<0.005$. $*p<0.05$; $**p<0.01$ (compared with control).

receptors. Mice were separated into individual cylindrical cages, with grids, for 15 min before the injection of apomorphine. Immediately after the injection, they were returned to the cages, and then their climbing behavior was observed for 5 seconds every 2 min. This behavior was scored as follows: four paws on the floor (0); holding onto the grids of the cage with forepaws (1); intermittent climbing (2); essentially uninterrupted climbing (3). Data was accumlated from 15 consecutive observations.

Biochemistry

The level of 3MT was measured to assess the amount of DA released from dopaminergic nerve terminal [10]. Fifteen minutes after the injection of apomorphine, the mice were killed by immersing into dry ice-ethanol solution to minimize postmortem increase of 3MT in the brain. The striatum was isolated from the nearly frozen brain on a cooled glass plate and stored at −80°C until assay for 3MT. The level of 3MT was measured by our assay procedure previously described [13]. The frozen striatum was homogenized with 150 μl of 0.1 N perchloric acid containing 0.1 mM EDTA, and then centrifuged for 10 min at 12,000 g (4°C). Thirty μl of a clear supernatant was injected into the HPLC coupled with electrochemical detection. The HPLC and detection system consisted of an HPLC pump (L-5000, Yanagimoto Company, Kyoto, Japan), a C-18 RP column (ODS-A, 25 cm × 4.6 mm, Yanagimoto) and a glassy carbon detector (VMD-501, Yanagimoto) set at a potential of 0.83 volts vs. the reference electrode. The composition of the HPLC buffer was 0.05 M sodium acetate/citric acid, pH 3.91, containing 16% (v/v) methanol, 0.1 mM EDTA and 1.63 mM heptanesulfonic acid. The flow rate of the HPLC was maintained at 1.1 ml/min.

Drugs

Apomorphine HCl (Sigma, St. Louis, MO) was dissolved in a saline solution containing 0.1% ascorbic acid. Haloperidol (Shionogi and Co., Ltd., Osaka, Japan) was dissolved in 0.1 M tartaric acid to a concentration of 1 mg/ml, and further diluted with distilled water for injection. Drugs were always injected at a volume of 0.05 ml/10 g body weight. The dosages of drugs were adapted from preliminary experiments and other reports [12].

Statistics

Data was analyzed by a single factor analysis of variance (ANOVA) and subsequently with Duncan's test for individual groups. In all statistical evaluation, $p<0.05$ was used as the criterion for statistical significance.

RESULTS

After injection of 0.2 mg/kg of apomorphine, cage climbing behavior score (climbing score) in the group pretreated with saline was significantly smaller than that in the control, while climbing score in the group pretreated with repeated administration of apomorphine did not differ from that in the control (Fig. 1. A). The 3MT level in the striatum of group pretreated with saline was significantly less after the injection of 0.2 mg/kg of apomorphine than that in the control, while the level of 3MT in the group pretreated with repeated administration of apomorphine did not differ from that of the control (Fig. 1. B).

After injection of 1.0 mg/kg of apomorphine, climbing score in the group pretreated with haloperidol was significantly higher than that in the group pretreated with saline. This shows that haloperidol was able to induce a state of supersensitivity to postsynaptic DA receptors, and 1 mg/kg of apomorphine stimulated postsynaptic DA receptors. With an injection of 0.2 mg/kg of apomorphine, climbing score in both groups was significantly smaller than that in the control (Fig. 2).

DISCUSSION

The results show that cage climbing behavior in the mice decreased with an acute low dose of apomorphine, while tolerance to apomorphine developed after repeated administration. Similar results (diminution of the activity-depressing effect of a low dose of apomorphine) have been observed in rats [1, 5, 11]. It is of interest to clarify why the activity-depressing effect of apomorphine decreases after repeated administration.

It is known that the repeated administration of neuroleptics results in a supersensitivity of postsynaptic DA receptors. This supersensitivity is manifested by a behavioral hypersensitivity to apomorphine [17]. In our experiment, climbing behavior induced by apomorphine at 1.0 mg/kg significantly increased after a repeated administration of haloperidol. It shows that mice were in a state of supersensitivity after the repeated haloperidol, whereas the activity-depressing effect of a low dose of apomorphine was not influenced in spite of the repeated haloperidol. Although others have suggested that disuse supersensitivity of postsynaptic receptor may cause tolerance to the effects of low doses of apomorphine, our results indicate this is not the case [11].

It is suggested that single low dose of apomorphine preferentially stimulates presynaptic DA receptors and decreases the release of DA from the dopaminergic nerve terminals [13]. A decreased release of DA is closely and rapidly reflected by a decrease in the formation of 3MT, an O-methylated metabolite of DA, in the rat [18] and mouse brain [10]. Our results show that the level of 3MT in the mouse striatum decreased with a single low dose of apomorphine, whereas the decrease in the level of 3MT was not observed after repeated administration. This change in the level of 3MT correlated with the behavioral change. Although it is difficult to interpret such biochemical change, and to correlate these changes with behavioral alterations, our results suggest that subsensitivity of presynaptic DA receptors participates in behavioral tolerance of a low dose of apomorphine.

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REFERENCES

1. Bernardi, M. M., C. Scavone and R. Frussa-Filho. Differential effect of single and long-term amphetamine and apomorphine administration on locomotor activity of rats. *Gen Pharmacol* **17:** 465–468, 1986.
2. Burt, D. R., I. Creese and S. H. Synder. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science* **196:** 326–328, 1977.
3. Carlsson, A. Receptor-mediated control of dopamine metabolism. In: *Pre- and Post-Synaptic Receptors*, edited by E. Usdin and W. E. Bunney. New York: Marcel Dekker, 1975, pp. 49–63.
4. Costal, B., C. D. Marsden, R. J. Naylor and C. J. Pycock. Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-OHDA lesion of extrapyramidal and mesolimbic nuclei. *Brain Res* **123:** 89–111, 1977.
5. Davis, E. R., W. W. Sant and G. Ellison. Continuous low-level apomorphine administration induces motor abnormalities and hallucinogen-like behaviors. *Psychopharmacology (Berlin)* **85:** 1–7, 1985.
6. Ernst, A. M. Relationship between the action of dopamine and apomorphine and their O-methylated derivation upon the CNS. *Psychopharmacologia* **7:** 391–399, 1965.
7. Gianutsos, G., R. B. Drawbaugh, M. D. Hynes and H. Lal. Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. *Life Sci* **14:** 887–898, 1974.
8. Goodale, D. B., D. B. Rustelholz, J. B. Long, J. R. Flynn, B. Walsh, J. G. Cannon and T. Lee. Neurochemical and behavioral evidence for a selective pre-synaptic dopamine receptor agonist. *Science* **210:** 1141–1143, 1980.
9. Kehr, W., A. Carlsson, M. Lindqvist, T. Magnusson and C. Atack. Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. *J Pharm Pharmacol* **24:** 744–747, 1972.
10. Kehr, W. 3-Methoxytyramine and normethanephrine as indicators of dopamine and noradrenaline release in mouse brain in vivo. *J Neural Transm* **50:** 165–178, 1981.
11. Kinon, B. J., D. Merson and J. M. Kane. Effect of daily dose of chronic haloperidol and chronic apomorphine on behavioral hypersensitivity in the rat. *Psychopharmacology (Berlin)* **84:** 347–351, 1984.
12. Marcais, H., P. Protais, J. Costentin and J. C. Schwartz. A gradual score to evaluate the climbing behavior elicited by apomorphine in mice. *Psychopharmacology (Berlin)* **56:** 233–234, 1978.
13. Murai, S., H. Saitoh, Y. Masuda, H. Yoshida, C. H. Chen and T. Itoh. Simultaneous determination of monoamines, their precursors and metabolites in discrete mice brain by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). *Jpn J Pharmacol* **40:** Suppl, 255P, 1986.
14. Nagy, J. I., T. Lee, P. Seeman and H. C. Fibiger. Direct evidence for presynaptic and postsynaptic dopamine receptors in brain. *Nature* **274:** 278–281, 1978.
15. Protais, P., J. Costentin and J. C. Schwartz. Climbing behavior induced by apomorphine in mice: a simple test for the study of dopamine receptors in striatum. *Psychopharmacology (Berlin)* **50:** 1–6, 1976.
16. Tarsy, D. and R. J. Baldessarini. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
17. Walters, J. R. and R. H. Roth. Dopaminergic neurons: An in vivo system for measuring drug interactions with pre-synaptic receptors. *Naunyn Schmiedebergs Arch Pharmacol* **296:** 5–13, 1976.
18. Westrink, B. H. C. and S. J. Spaan. On the significance of endogenous 3-methoxythyramine for the effects of centrally acting drugs on dopamine release in the rat brain. *J Neurochem* **38:** 680–686, 1982.