Further Dopaminergic Supersensitivity to Dopamine Agonists by Subchronic Administration of Haloperidol in Rats Treated with Colchicine

Katsuo KAMATA, Shigeru OKUYAMA*, Sanae HASHIMOTO*,
Hironaka AIHARA* and Yutaka KASUYA

Department of Pharmacology, School of Pharmacy, Hoshi University, Tokyo 142, Japan

*Research Center, Taisho Pharmaceuticals, Saitama 330, Japan

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Abstract—Effects of subchronic administration of haloperidol on the circling behavior induced by dopamine agonists were examined in rats treated with colchicine. Methamphetamine and apomorphine produced contralateral or ipsilateral rotation in colchicine-treated rats, and these circling behaviors were significantly increased by subchronic treatment with haloperidol. These results suggest that development of supersensitivity following colchicine and subchronic haloperidol may occur through different mechanisms.

When colchicine, a drug which has been known to suppress axoplasmic transport, is directly applied to a nerve, axoplasmic transport is blocked, causing a denervation-like supersensitivity of skeletal (1–4) and smooth muscle (5–7). Recently, we have reported that denervation-like supersensitivity can be caused by the block of axoplasmic transport in the central nervous system (8). It has been reported by us that methamphetamine, an indirectly acting dopamine agonist, produces contralateral circling behavior and apomorphine produces an ipsilateral followed by a contralateral circling behavior in rats injected with colchicine into the substantia nigra pars compacta (SNC), and we reported that colchicine might produce changes in postsynaptic dopamine receptors by interrupting the fast axonal transport of trophic factor(s) as it is known to do in the peripheral adrenergic nerve fibers (9–11).

On the other hand, chronic administration of neuroleptics has been shown to produce a supersensitivity to dopamine agonists after these drugs are withdrawn (12–14). In these animals, dopamine receptor binding in the neostriatum is increased (15), and there is a corresponding increase in the behavioral

response to dopamine agonists (16–18). Furthermore, it has recently reported that the up-regulation of dopamine receptors in rat nucleus accumbens or caudate nucleus after denervation and receptor blockade is additive (19, 20).

In the present study, we investigated the effect of subchronic haloperidol on the denervation-like postsynaptic supersensitivity to dopamine agonists induced by micro-injection of colchicine into the SNC by using the circling behavioral model which has been established by Ungerstedt (21, 22). The purpose of the present study was to determine whether development of supersensitivity following the block of axoplasmic transport and that due to receptor blockade could arise from independent mechanisms.

All experiments were carried out on male rats of the Wistar strain weighing 200–300 g. The surgical procedure for lesioning of the SNC has been described previously (8, 23–25). Briefly, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), placed in a stereotaxic apparatus and subsequently subjected to the surgical operation as follows: Following exposure of the skull, a burr hole was drilled over the left SNC (approximately 2.8 mm anterior to lambda, 2.0 mm lateral to

the midline) according to the stereotaxic atlas of König and Klippel (26). A stainless-steel cannula attached to a microsyringe containing 20 $\mu\text{g}/\mu\text{l}$ colchicine in sterile saline was lowered 7.3 mm ventral to the dura. The cannula was kept in position for 5 min before a 1 μl volume of the solution of colchicine was injected at a rate of 0.5 $\mu\text{l}/\text{min}$. The cannula remained in place for an additional 5 min and then was slowly withdrawn from the brain. The wound area was closed, and the animal was injected intramuscularly with 10,000 units penicillin.

For measurement of the drug-induced circling behavior, rats were placed in individual testing cages (35 $\times$ 25 $\times$ 18 cm) and left undisturbed for adaptation to the cage for 1 hr. Circling behavior was directly observed for 2 hr and a 360° turn was considered as one circling count. The number of complete turns and the direction of circling were recorded for 5 min at 15, 30, 60, 90 and 120 min following the administration of drugs. Apomorphine HCl and methamphetamine HCl were administered intraperitoneally as a salt. Circling responses to methamphetamine (Dainippon Phar-

maceuticals) (2.0 mg/kg) or apomorphine (Sigma) (1.0 mg/kg) were determined 8 days following the surgical operations. One day after surgery, animals were pretreated with 2 daily subcutaneous injections of saline (1.0 ml/kg) or 1.0 mg/kg haloperidol (Dainippon Pharmaceuticals) for 7 days. At the end of the behavioral experiments, the animals were sacrificed under deep pentobarbital sodium anesthesia and perfused with 10% formalin. Frozen 50 μm thick sections of the whole brain were cut using a freezing microtome. The location of each lesion site was verified. Only rats in which the lesion target was confirmed histologically were included in the analysis of the behavioral data. Student's *t*-test was used for statistical evaluation of the data. Less than 0.05 level of probability was accepted as significant.

Consistent with previous report (8), unilateral injection of colchicine (20 $\mu\text{g}/\text{animal}$) into the SNC produced animals that showed a slight occasional bias in their posture toward the injected side and became aggressive 3–8 days after the treatment. As shown in Fig. 1, rats injected with colchicine into the SNC rotated toward the side of

Fig. 1. Circling behavior induced by apomorphine (A) and methamphetamine (B) in rats microinjected with colchicine into the SNC. Drugs were given 8 days following infusion of colchicine. Each symbol, (closed circle, subchronic haloperidol-treated rats; open circle, subchronic saline-treated rats), represents the mean $\pm$ S.E. of 10–12 determinations. * $P < 0.05$ vs. saline, ** $P < 0.01$ vs. saline. Contralateral circling is indicated by positive numbers (upward direction), and ipsilateral circling is indicated by negative numbers (downward direction).

infusion after 1.0 mg/kg of apomorphine (ipsilateral circling: indicated by negative numbers and a downward direction) and away from the side of infusion after 2.0 mg/kg of methamphetamine (contralateral circling: indicated by positive numbers and an upward direction). The characteristic response to both drugs was tight, 'nose-to-tail' turning. Both apomorphine and methamphetamine-induced ipsilateral or contralateral circling behaviors were significantly increased by treatment with subchronic haloperidol as shown in Fig. 1.

In the present experiments, methamphetamine produced contralateral circling behavior in rats injected with colchicine into the SNC. It has been recently reported that postsynaptic dopamine receptors in the striatum ipsilateral to the injection side appear to be supersensitive to the released dopamine, and colchicine might produce changes in postsynaptic dopamine receptors by interrupting the influence of some neurotrophic factor(s); e.g., trophic factor(s) via an inhibitory action of the fast axonal transport system as it is known to do in the peripheral adrenergic nerve fibers (9–11). Chronic haloperidol treatment produced further supersensitivity, indicating that development of supersensitivity following colchicine and subchronic haloperidol involve different regulatory processes; i.e., these treatment can lead to independent processes of postsynaptic supersensitivity. Of particular interest in this connection is the observation that in animals with bilateral injections of 6-hydroxydopamine into the nucleus accumbens and chronic haloperidol administration, there is a greater increase in the density of ^3H -spiperone-binding sites than there is with either treatment alone, and the affinity constant is not altered (19, 20).

On the other hand, apomorphine produced an ipsilateral circling behavior on day 8 in rats injected with colchicine into the SNC. Apomorphine produces contralateral circling in unilaterally 6-hydroxydopamine-denervated rats (21, 22). Herrera-Marschitz and Ungerstedt (27) demonstrated that the contralateral apomorphine rotation is converted into ipsilateral turning following kainic acid lesion of the substantia nigra, although con-

tralateral circling is maintained. They suggested that this conversion of apomorphine-induced contralateral rotation into ipsilateral circling is mainly dependent upon striatonigral pathways. It is conceivable, therefore, that apomorphine-induced ipsilateral circling in colchicine-treated rats may be responsible for striatonigral pathways on the intact side. Chronic treatment with haloperidol enhanced apomorphine-induced ipsilateral circling, indicating that chronic haloperidol might produce the enhancement of striatonigral pathways as suggested by Gale (28) and Huffman and Tick (29). They reported that the chronic administration of haloperidol resulted in a significant increase in binding of GABA to receptors in the substantia nigra. This idea is one of various possibilities. With respect to the detailed mechanism concerning the enhancement of apomorphine-induced ipsilateral circling by subchronic haloperidol in colchicine-treated rats, further investigations are required.

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