EFFECTS OF BILATERAL LESIONS IN THE STRIATUM OR NUCLEUS ACCUMBENS ON THE CATALEPTOGENIC ACTIVITY OF NEUROLEPTICS IN RATS

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Abstract—To investigate the role of the striatum and nucleus accumbens in neuroleptic-induced catalepsy, bilateral electrocoagulations were made or microinjections of 6-hydroxydopamine (6-OHDA) were given to rats in these brain regions, and the cataleptogenic activity of neuroleptics was measured. Electrocoagulation in these regions caused a highly specific destruction of brain tissue, and 6-OHDA decreased the levels of dopamine in the injected region with little effect on these levels in other regions. The cataleptogenic activity of haloperidol was enhanced by the electrocoagulation in the striatum at 2 days after the operation, but was weakened from 7 days on. The electrocoagulation weakened the catalepsy induced by chlorpromazine, thioridazine, and ID-4708 (a new butyrophenone derivative), but enhanced that by clozapine at 2 weeks after the operation. Microinjection of 6-OHDA into the striatum enhanced the catalepsy induced by the five neuroleptics used. The lesions in the nucleus accumbens had fewer effects on catalepsy than did those in the striatum. It was concluded that the striatum more than the nucleus accumbens is involved in producing catalepsy with neuroleptics, and that the enhancement of catalepsy by electrocoagulation in the striatum is characteristic of clozapine.

Pharmacological, biochemical and electrophysiological investigations showed that neuroleptics block dopamine (DA) receptors in the brain (1-3). Almost all neuroleptics produce catalepsy in rats. Reserpine and tetrabenazine reduce the monoamine contents and produce catalepsy (4, 5). These facts suggest that the catalepsy is the result of blockade of DA receptors or the reduction of the activity of DA neurons (6). DA containing nerve terminals are localized in the striatum and nucleus accumbens (7). Therefore, the catalepsy may be induced by the effect of drugs on these brain regions. The present study was undertaken to investigate the contribution of DA neurons in the striatum (extrapyramidal system) and nucleus accumbens (mesolimbic system) to the catalepsy induced by neuroleptics. There are data as to the effects of electrocoagulation in the caudate-putamen on the cataleptogenic activity of several neuroleptics (8, 9). We investigated the effects of electrocoagulation in the striatum or nucleus accumbens on the cataleptogenic activity of haloperidol, ID-4708 (a new butyrophenone derivative), chlorpromazine, thioridazine and clozapine. Effects of microinjection of 6-hydroxydopamine (6-OHDA) into the striatum or nucleus accumbens on the cataleptogenic activity of these five neuroleptics were also investigated.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 150–170 g at the time of operation, were allowed free access to food and water except during the experiments. Rats were housed in groups at $25 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity.

Microinjection of 6-OHDA into the striatum or nucleus accumbens

Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and placed on a standard stereotaxic instrument (Takahashi Shōten). The skull was exposed. A stainless steel cannula (0.3 mm in outer diameter) was attached to the stereotaxic instrument and placed on a predetermined position on the skull. A small opening was made with a dental drill. The injection cannula was inserted into the brain tissue through this opening. The solution of 6-OHDA was injected through the injection cannula which was connected with a motor-driven microsyringe (Stoelting Co., U.S.A.) Three injections in each side of the striatum of each animal were given with anterior, lateral and vertical coordinates 6.8, 3.5, ± 0.0 ; 7.5, 2.5, $+0.5$; 8.4, 2.6, $+1.0$ according to the brain atlas of König and Klippel (10). The coordinates used for the bilateral injections of 6-OHDA into the nucleus accumbens were 9.4, 1.0, -0.9 . A 4 μg of 6-OHDA was dissolved in 2 μl of physiological saline containing 0.1% ascorbic acid and was injected into the brain tissue at the rate of 1 $\mu\text{l}/\text{min}$. After the injection of 6-OHDA, the injection cannula was left in the place for 1 min to allow for adequate diffusion of the solution. Total amounts of 6-OHDA injected to each rat were 24 μg into the striatum or 8 μg into the nucleus accumbens. Sham operations were made by the injection of the same volume of the vehicle into the brain tissue in the same way as used for the injection of 6-OHDA. DA contents were measured according to the method of Chang (11) at the end of experiments.

Electrocoagulation in the rat brain

Rats were anesthetized with sodium pentobarbital and placed on a standard stereotaxic instrument. Electrocoagulation was caused by passing a current of 0.5 mA for 30 min through a bipolar indwelling stainless steel electrode (0.5 mm in diameter). The coordinates used for the coagulation in the striatum or nucleus accumbens were the same as used for the injection of 6-OHDA into these regions. Sham operations were performed in exactly the same way as used in the electrocoagulation except that no current was passed through the electrode. Size of the coagulated area was measured in histological examinations at the end of experiments. The rats were again anesthetized with pentobarbital and perfused with saline through the heart. After blood was washed out, the brains were fixed with Bouin's solution, and then cut in 100 μm thick sections, stained with hematoxylin and eosin, and the size of coagulated areas measured.

Assessment of catalepsy

The intensity of catalepsy was measured according to the method of Wirth et al. (12) with slight modification. Both front paws of rats were placed on a horizontal metal bar, 8.8 cm in height, and the rats were forced to rest on hind legs only. The duration (in sec)

of this abnormal position was regarded as a catalepsy score. When catalepsy lasted for more than 300 sec, the catalepsy score was regarded as 300.

Drugs

All neuroleptics used were micronized prior to use and suspended or dissolved in 5% gum arabic solution before the administration. Haloperidol, clozapine and ID-4708, a new butyrophenone derivative (13), were synthesized in our laboratory. Chlorpromazine (Yoshitomi Seiyaku), thioridazine (Sankyo) and 6-hydroxydopamine hydrobromide (Sigma) were commercially purchased. Dosage of the drug is expressed in terms of base.

Statistical evaluation

The catalepsy score was compared between control and operated groups using the Mann-Whitney *U*-test.

RESULTS

Time-course of the effect of lesions in the striatum or nucleus accumbens on the cataleptogenic activity of haloperidol

The cataleptogenic activity of haloperidol (2 mg/kg s.c.) was measured in each rat at

FIG. 1. Effects of bilateral electrocoagulations in the striatum or nucleus accumbens and bilateral injections of 6-OHDA into the striatum or nucleus accumbens on the intensity of catalepsy induced by haloperidol in rats. The cataleptogenic activity of haloperidol (2 mg/kg s.c.) was measured at 2, 7, 14 and 21 days after the operation. The catalepsy score was measured at 6 hr after the administration, and the means and S.E.M. were calculated. ○—○: unoperated control (N=7). □- -□: microinjection of 6-OHDA into the striatum (N=8). ■- · -■: microinjection of 6-OHDA into the nucleus accumbens (N=8). △ △: electrocoagulation in the striatum (N=7). ▲- · -▲: electrocoagulation in the nucleus accumbens (N=8). Vertical bars represent S.E.M. Statistical comparisons between control and operated rats were performed with *U*-test. **P*<0.05. ***P*<0.01. ****P*<0.001.

2, 7, 14 and 21 postoperative days. The intensity of catalepsy is shown in Fig. 1. The cataleptogenic activity of haloperidol was not altered by sham operations at any stage after the operation.

In rats given 6-OHDA into the striatum, the cataleptogenic activity of haloperidol gradually increased in accordance with postoperative days. The injection of 6-OHDA into the nucleus accumbens did not affect the catalepsy induced by haloperidol.

The electrocoagulation in the striatum caused a marked enhancement of the catalepsy induced by haloperidol at 2 days after the operation. However, the cataleptogenic activity of haloperidol in operated rats markedly decreased compared to control rats at 7, 14 and 21 days after the operation. The electrocoagulation in the nucleus accumbens enhanced the catalepsy induced by haloperidol at 2 days and weakened it at 7 days or later after the operation.

Effects of lesions in the striatum or nucleus accumbens on the cataleptogenic activity of neuroleptics

Each group of 6 rats was subjected to 6-OHDA or electrocoagulation in the striatum or nucleus accumbens, and then given drugs 2 weeks after. Each rat underwent only one trial of drug administration. The intensity of the catalepsy induced by haloperidol, ID-4708, chlorpromazine, thioridazine and clozapine in rats is shown in Fig. 2. The cataleptogenic activity of neuroleptics was not altered by sham operations.

Microinjection of 6-OHDA into the striatum remarkably enhanced the catalepsy induced by all neuroleptics tested in this experiment. The cataleptogenic activity of thioridazine and clozapine was significantly enhanced by the injection of 6-OHDA into the nucleus accumbens, but the enhancement was not so great as that by the injection of 6-OHDA into the striatum. The cataleptogenic activity of chlorpromazine was enhanced only at 1 h after the administration but that of haloperidol was not enhanced by the 6-OHDA lesion of the nucleus accumbens.

The electrocoagulation in the striatum markedly weakened the cataleptogenic activity of haloperidol, ID-4708, chlorpromazine and thioridazine, whereas enhanced that of clozapine. The electrocoagulation in the nucleus accumbens weakened the catalepsy induced by thioridazine and chlorpromazine, whereas enhanced that by clozapine. The influence of electrocoagulation in the nucleus accumbens was less than that of electrocoagulation in the striatum.

Decrease in DA content after injection of 6-OHDA and the degree of electrocoagulation

The injection of 6-OHDA into the striatum decreased the DA levels in the striatum and nucleus accumbens to 20 ± 5 (mean $\pm$ S.E.M.) and $56 \pm 8\%$ ($N=38$) of those of unoperated control rats, respectively. The injection of 6-OHDA into the nucleus accumbens decreased the DA levels in these two regions to 92 ± 6 and $12 \pm 2\%$ ($N=38$) of control rats. The DA contents of the striatum and nucleus accumbens in control rats were 8.62 ± 0.75 and 7.88 ± 0.51 $\mu\text{g/g}$ tissue ($N=37$), respectively. Sham operations did not affect the DA levels in these regions.

Histological examinations showed that the electrocoagulation in the striatum destroyed 60–80% of the striatum and 5–10% of the nucleus accumbens (N=37). The electrocoagulation in the nucleus accumbens caused a destruction of 90–100% of the nucleus accumbens and 5–10% of the striatum (N=38).

FIG. 2. Effects of bilateral lesions in the striatum or nucleus accumbens on the catalepsy induced by 1 mg/kg of haloperidol (HPD), 1 mg/kg of ID-4708 (4708), 10 mg/kg of chlorpromazine (CPZ), 30 mg/kg of thioridazine (TRZ) and 30 mg/kg of clozapine (CLOZ). All drugs were given subcutaneously. Catalepsy was measured at 2 weeks after the operation. Mean catalepsy score of each group of 6 rats was calculated. ○---○: unoperated control. □---□: microinjection of 6-OHDA into the striatum. ■---■: microinjection of 6-OHDA into the nucleus accumbens. △---△: electrocoagulation in the striatum. ▲---▲: electrocoagulation in the nucleus accumbens. *P<0.05.

DISCUSSION

Histological and biochemical experiments showed that the electrocoagulation in bilateral striata and nucleus accumbens caused a highly specific destruction of these brain regions, and that the microinjection of 6-OHDA into one of these regions decreased the DA content in the injected region with little effect on DA in other regions.

The electrocoagulation in the striatum enhanced the catalepsy induced by haloperidol at 2 days after the operation, but decreased it at 7 days or later. It is well known that catalepsy is induced not only by drugs which decrease the dopaminergic transmission but also by the cholinomimetic drugs. The nigrostriatal dopaminergic neurons exert a direct inhibitory influence on the activity of striatal cholinergic neurons (14–16). As to the mechanism of catalepsy, the simplest interpretation is that the catalepsy is induced by the direct stimulation of cholinergic receptors with cholinomimetics or by the excitation of cholinergic neurons through the blockade of inhibitory activity of dopaminergic neurons with neuroleptics. The electrocoagulation must have destroyed not only DA neurons but also cholinergic neurons. Rats subjected to electrocoagulation in the striatum showed a cataleptic state without drug administration at 2 days after the lesion (data not included). The activity of the neurons innervated from cholinergic sources may be temporarily and compensatorily high at 2 days after the lesion, and if so, such would explain the enhancement of haloperidol-induced catalepsy at 2 days after the lesion. However, at 7 days or later after the lesion, the destruction of dopaminergic-cholinergic connections was complete, and such would cause a reduction in the cataleptogenic activity of haloperidol. On the other hand, the microinjection of 6-OHDA into the striatum gradually enhanced the catalepsy induced by haloperidol. With the administration of neuroleptics, the release of DA from the nerve terminals in the striatum is accelerated probably through a feedback mechanism (1). When the DA stored in the nerve terminals is decreased by 6-OHDA, the acceleration of DA release may be interrupted and such would lead to decrease in the activity of DA neurons. Thus, the cataleptogenic activity of neuroleptics seems to be enhanced after the microinjection of 6-OHDA. The injection of 6-OHDA into the striatum produced slight catalepsy without drug administration, and the catalepsy was not so marked as that induced by reserpine or tetrabenazine (unpublished). The decrease in dopaminergic transmission induced by 6-OHDA would be compensated for by the supersensitivity of the DA receptors (17).

The lesion in the striatum caused more marked effect on the catalepsy induced by five neuroleptics tested in this experiment than that in the nucleus accumbens. This fact suggests that the striatum is important in regulating the degree of catalepsy and that the nucleus accumbens may play a limited role in regulating catalepsy. The striatal injection of 6-OHDA enhanced the catalepsy induced by the five neuroleptics used. The electrocoagulation in the striatum markedly reduced the cataleptogenic activity of haloperidol, ID-4708, chlorpromazine and thioridazine, but enhanced that of clozapine. Other workers have shown that the bilateral electrocoagulations in caudate-putamen reduced the catalepsy induced by perphenazine or haloperidol, whereas enhanced that by clozapine at 2 weeks or later after the operation (8, 9). Our findings are in good agreement. Thus, the possibility

that the mechanism of catalepsy induced by clozapine is entirely different from that by other four neuroleptics has to be considered.

ID-4708 was similar to haloperidol and chlorpromazine regarding effects of electrocoagulation in the striatum or nucleus accumbens and microinjection of 6-OHDA into these regions. These results show that the mechanism of catalepsy induced by ID-4708 is similar to that induced by haloperidol or chlorpromazine.

It is concluded that the striatum plays a greater role in the production of catalepsy with neuroleptics than does the nucleus accumbens, and that the enhancement of catalepsy by the electrocoagulation in the striatum is characteristic of clozapine.

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