EFFECTS OF CDP-CHOLINE ON STRIATAL DOPAMINE LEVEL AND BEHAVIOR IN RATS

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Abstract—To further assess the effects of CDP (cytidine diphosphate)-choline on Parkinsonian symptoms, striatal dopamine (DA) was measured fluorometrically in rats after injection of CDP-choline. CDP-choline (300 mg/kg, i.p.) increased the DA content in the striatum (p<0.05) one hour after injection. The behavioral effect of CDP-choline was then tested in rats in which the unilateral nigro-striatal DA neurons had degenerated following an intranigral injection of 6-hydroxydopamine (6-OHDA). CDP-choline alone did not produce behavioral changes in these rats. However, pretreatment with a single dose of CDP-choline (900 mg/kg, i.p.) suppressed both the apomorphine-induced contralateral and the d-amphetamine-induced ipsilateral circling. The same dose of CDP-choline suppressed the number of treadmill revolutions in mice. On the other hand, a 7-day consecutive treatment with 300 mg/kg of CDP-choline enhanced the apomorphine-induced contralateral circling (by 42%, p<0.05). The same treatment with CDP-choline raised the striatal DA content by 29% (p<0.05) on the intact side, but not on the 6-OHDA injected side.

These results indicate that CDP-choline has neither a direct nor an indirect DA agonistic effect. The increase in DA content, decrease in locomotion and enhancement of the effect of apomorphine can be explained on the hypothesis that CDP-choline may act as an antagonist on the DA neurons and receptors. The validity of this apparently paradoxical use of CDP-choline with antagonistic effect on DA neurons in the treatment of Parkinson’s disease is discussed.

Effectiveness of CDP-choline (cytidine diphosphate choline) on symptomatology of Parkinson's disease is unanimously attributed to its dopamine (DA) elevating action (1). However, current pharmacological findings indicate that DA agonists decrease DA synthesis while DA antagonists increase DA synthesis and turnover rates (2, 3). Both mechanical injury to nigrostriatal DA pathway and administration of gamma-butyrolactone (GBL) are known to raise striatal DA levels (4, 5). Thus DA elevating action of CDP-choline may indicate its antagonistic effect on DA-nergic mechanism.

To analyse the pharmacological effects of CDP-choline on the nigrostriatal DA neurons, the striatal DA level was measured after injection of CDP-choline into intact rats, and behavioral effects of CDP-choline, alone or in combination with apomorphine or d-amphetamine, were observed in rats in which unilateral nigrostriatal DA neurons had
been destroyed by injection of 6-OHDA into the substantia nigra.

MATERIALS AND METHODS

Male 200 g Wistar rats were used for all experiments except in cases of studies of locomotion in which adult mice were used.

Striatal dopamine level: The dose response relationship (50–1200 mg/kg, i.p.) and the time course (0.5–6 hrs) of the effect of CDP-choline on striatal dopamine (DA) levels were studied in intact rats. The effect of repeated doses of (once a day for 7 days) of CDP-choline was examined in 6-OHDA treated rats. The animals were decapitated at the time indicated and the corpus striatum was dissected quickly and stored at -80°C until use. DA was extracted from tissue homogenates according to the method of Anton and Sayre (6) and assayed fluorometrically (7).

Behavioral observations: Behavioral effects of CDP-choline were studied in 6-OHDA treated rats and intact mice. 6-OHDA (8 μg/4 ul saline with 0.1% ascorbic acid) was stereotaxically (Takahashi Medical Inst. Co., Tokyo) injected unilaterally into the substantia nigra, as previously described (8). Over one month after 6-OHDA administration, degeneration of the nigrostriatal DA neurons on the injected side was evident by ipsilateral circling (more than 4 turns per min) after administration of d-amphetamine (3 mg as base/kg, i.p.) and also by contralateral circling after apomorphine (1 mg as base/kg, i.p.). CDP-choline (300 and 900 mg/kg) was given i.p. to these DA neuron impaired rats 30 min before treatment with apomorphine or d-amphetamine. The number of circlings was counted at 30 min after injection of apomorphine or d-amphetamine and the results were compared with those of apomorphine or d-amphetamine alone. In another group, CDP-choline (100 and 300 mg/kg, i.p.) was injected once a day for 7 consecutive days. The number of circlings of these rats after injection of apomorphine or d-amphetamine was compared with the number in rats given a single dose of CDP-choline. In the 7-day treatment with CDP-choline, apomorphine or d-amphetamine was injected 30 min after the last dose of CDP-choline.

Effects of CDP-choline on locomotion were compared with the number of revolutions made by the saline-injected mice. Statistical analysis was made using the two-tailed t-test.

Drugs used were as follows: CDP-choline (Takeda), dopamine hydrochloride (Nakarai), 6-hydroxydopamine hydrochloride (Sigma), apomorphine hydrochloride (McFarlan Smith), d-amphetamine sulfate (Dainippon). All other chemicals were of analytical grade and the best commercially available.

RESULTS

Intact rats: The dose-response relationship of CDP-choline (one hour after i.p. administration) on the striatal DA level in intact rats is shown in Fig. 1. The DA level tended toward an increase with increase in the dose of CDP-choline and a significant change was observed at 900 mg/kg.

![Fig. 1. The dose response relationship of CDP-choline on striatal dopamine level. Rats were sacrificed one hour after CDP-choline injection. Bars indicate mean±S.E.M. Shaded area indicates the range of the control value (7.13±0.28 μg/g, N=15). Number of rats is shown on top of columns. *: p<0.05.](image-url)
(p<0.05) elevation was seen with the doses of 300 and 1200 mg/kg (by up to 23% above control value). The increase in striatal DA after 300 mg/kg of CDP-choline was significant one hour later and returned to the control level 3 hours later (Fig. 2). The increase in DA content after the same dose of L-DOPA was considerably higher (up to 74% over the control) and lasted longer than that after administration of CDP-choline (Fig. 2). There was no significant change in DA and noradrenaline (NA) content in the whole brain one hour after the doses of 100 and 300 mg/kg of CDP-choline (data not shown).

6-OHDA injected rats: CDP-choline alone (300 and 900 mg/kg, i.p.) did not produce behavioral changes in rats in which the nigro-striatal DA neurons had been unilaterally destroyed by intranigral administration of 6-OHDA, while apomorphine (1 mg/kg, i.p.) and d-amphetamine (3 mg/kg, i.p.) induced circling in the contralateral and ipsilateral directions, respectively. Although a single dose of 300 mg/kg of CDP-choline 30 min prior to administration of apomorphine or d-amphetamine did not modify the effects of the latter agents, increasing the dose of CDP-choline to 900 mg/kg resulted in a significant suppression of both the apomorphine-induced contralateral and d-amphetamine-induced ipsilateral circling (Fig. 3). In contrast to the single dose, repeated injections for 7 days of CDP-choline (300 mg/kg) in 6-OHDA treated rats increased the apomorphine-induced contralateral circling by 42% (p<0.05) of control value. Such was not the case after repeated doses of 100 mg/kg of CDP-choline. The d-amphetamine-induced circling was not significantly altered by repeated treatment with 100 and 300 mg/kg of CDP-choline (Fig. 3). When the assay of striatal DA was
Table 1. Effects of CDP-choline on striatal dopamine levels in 6-OHDA injected rats (n=5)

|                | Without CDP-choline | CDP-choline (300 mg/kg/day) for 7 days |
|----------------|---------------------|----------------------------------------|
| Dopamine (µg/g) |                     |                                        |
| Control side   | 7.14±0.39           | 9.22±0.81*                             | p<0.05 |
| 6-OHDA SIDE    | 0.55±0.23           | 0.32±0.18 NS                           |

Measurement was made 1 hr after the last injection of CDP-choline.

DISCUSSION

CDP-choline produced a mild increase in the DA level in the striatum and the increase was prevented by the injection of 6-OHDA in the nigra, indicating that this drug may promote synthesis and/or uptake of DA or may inhibit the release of DA from the DA neuron. Such findings are inconsistent with the results reported by Manaka et al. (1) who found an increase in striatal DA on the lesioned side in cats. One reason for the discrepancy may be the difference in the method of making lesions in the nigrostriatal DA system. The latter investigators used a radiofrequency coagulation which would destroy indiscriminately various nerve components including the DA neuron system. Another reason for the discrepancy may be due to the difference in the time of CDP-choline administration with respect to the degeneration of nigrostriatal DA neurons. Manaka et al. (1) started CDP-choline administration soon after the stereotaxic surgery when degeneration was still progressing while we used the drug later than one month after 6-OHDA administration when degeneration was probably established.

CDP-choline produced no circling behavior in 6-OHDA treated rats which had responded
with contralateral turning to apomorphine and with ipsilateral turning to d-amphetamine, indicating that CDP-choline has neither apomorphine-like "direct" nor d-amphetamine-like 'indirect DA agonistic effects'. Yet a high dose of CDP-choline exerted a sedative effect on spontaneous locomotion in mice.

Why apomorphine-induced contralateral circling was enhanced by repeated injections of CDP-choline remains unknown. As the experiments were carried out over one month after injection of 6-OHDA when denervation supersensitivity was probably in a steady state (9), it is unlikely that this enhancement is the result of development of denervation effect during the one week treatment with CDP-choline. Whether this enhanced effect of apomorphine is due to an increase in the affinity of DA receptors or to an increase in the number of DA receptors remains to be determined. Chronic treatment with neuroleptics enhances the effect of apomorphine (10) and increases the number of DA binding sites in the striatum (11). Neuroleptics also raise the DA turnover rate by interrupting the inhibitory feedback mechanism (2). There is one known DA antagonist which raises DA levels in the striatum (5).

Thus, our finding that CDP-choline produces an increase in DA levels, decrease in locomotion, suppression of apomorphine- and d-amphetamine induced circling with a single dose and enhancement of the effect of apomorphine with repeated injections, can be explained on the basis of the idea that CDP-choline may act as an antagonist on the DA receptor system. However, the clinical effectiveness of CDP-choline in the treatment of Parkinson's disease may occur under circumstances in which repeated administration of L-DOPA decreases the sensitivity of DA receptors and produces a tolerance to L-DOPA. CDP-choline may act on the DA receptor and reverse the lowered sensitivity of the DA receptor to the normal.

As the dose of CDP-choline used in our experiments was relatively high and was given only for a short period, the effect of smaller doses and longer periods of administration should be evaluated in order to determine the effectiveness of CDP-choline in the clinical treatment of Parkinson’s disease.

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