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Potentiation by Deprenil of l-Dopa Induced Circling in Nigral-Lesioned Rats

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HEIKKILA, R. E., F. S. CABBAT, L. MANZINO AND R. C. DUVOISIN. *Potentiation by deprenil of l-dopa induced circling in nigral-lesioned rats.* PHARMAC. BIOCHEM. BEHAV. **15**(1) 75–79, 1981.—l-Deprenil, a potent inhibitor of type B monoamine oxidase, was a weak inhibitor of ^{3}H-dopamine uptake as well as a weak releasing agent for previously accumulated ^{3}H-dopamine in rat neostriatal tissue slices. In similar experiments d-amphetamine was approximately 100 times as potent as l-deprenil as a releasing agent. When deprenil (20 mg/kg IP) was given to rats with a unilateral 6-hydroxydopamine lesion of the substantia nigra, it brought about a moderate but long-lasting ipsilateral rotational behavior. l-Dopa (20–40 mg/kg, IP) by itself caused a considerably stronger rotation in the opposite direction (contralateral). When l-dopa was given to rats 1 hr after l-deprenil, there was a considerably greater contralateral rotation than after l-dopa alone. Clorgyline, a type A monoamine oxidase inhibitor, which by itself at 20 mg/kg caused no rotation, also potentiated the contralateral rotation seen after l-dopa (5–20 mg/kg). In contrast, d-amphetamine, which by itself caused ipsilateral rotation, failed to potentiate the rotation after l-dopa. Possible mechanisms for these observations will be discussed.

l-Deprenil Clorgyline l-Dopa Parkinsonism ^{3}H-dopamine release ^{3}H-dopamine uptake

IT is now well-accepted that there are multiple forms of the enzyme monoamine oxidase (MAO) distinguished by different substrate specificities [13,17]. Moreover, inhibitors of MAO may be classified according to their ability to inhibit the deamination of various substrates [17]. For example, it has been shown that phenylethylamine is deaminated primarily by MAO-B in rodents and that deprenil is a relatively specific inhibitor of MAO-B [6, 9, 17]. In contrast, it has been observed that serotonin is deaminated in rodents primarily by MAO-A and that clorgyline is a relatively specific inhibitor of MAO-A [17]. Interestingly, dopamine is thought to be a substrate for both forms of the enzyme [5, 15, 17]. If this were so, then one would predict that either inhibitors of MAO-A or of MAO-B should enhance the behavioral effects of l-dopa in rodents.

It has been shown that deprenil administration to rats results in a large elevation in central dopamine levels [4]. Moreover, it is felt that dopamine is almost exclusively a substrate for MAO-B in humans [6]. For these reasons deprenil has been tried in the treatment of Parkinson's disease, in which there is a well-defined dopamine deficiency, with the hope that deprenil administration would raise brain levels of dopamine by preventing its deamination (inactivation).

The present study was undertaken with a view towards clarifying the mode of action of deprenil. Its effects were studied on l-dopa induced rotational behavior in rats with a unilateral 6-hydroxydopamine lesion of the substantia nigra. These lesioned animals have been widely used as an animal model for Parkinson's disease and as a test system for potential anti-Parkinsonian agents. In addition, the capacity of deprenil to cause the release of or to block the uptake of ^{3}H-dopamine in rat neostriatal tissue slices was determined. This was done because deprenil is a close structural analog of amphetamine, a relatively potent releasing agent for dopamine. Furthermore, it has been shown that deprenil is metabolized in vivo to amphetamine and to methamphetamine [12]. Amphetamine by itself causes intense ipsilateral rotation in the nigral-lesioned rat whereas l-dopa causes intense contralateral rotation [14]. Our data show that deprenil is a weak ^{3}H-dopamine uptake inhibitor and a weak releasing agent for previously accumulated ^{3}H-dopamine in neostriatal tissue slices. Moreover, the data will show deprenil causes a moderate ipsilateral rotation in rats with a 6-OHDA lesion of the substantia nigra, but greatly enhances the contralateral rotation induced by l-dopa.

METHOD

Rotation in 6-Hydroxydopamine-Lesioned Rats

Female Sprague-Dawley rats weighing 150–175 g were subjected to a unilateral 6-hydroxydopamine lesion performed essentially as described [14] with minor modifica-

tions. A solution of 6-hydroxydopamine HBr containing 8 μg in 4 μl of 0.2 mg/ml ascorbic acid was injected into the rostromedial portion of the left substantia nigra of rats under Brevital anesthesia (Rx, Lilly, Indianapolis, IN) in a David Kopf model 900 stereotaxic apparatus. Desipramine·HCl (Ciba-Geigy, Summit NJ) at 25 mg/kg was administered 0.5 hr prior to 6-hydroxydopamine to protect noradrenergic terminals. Lesion coordinates derived from the König-Klippel atlas [10] were A 2.6 mm, L 1.8 mm and V −2.6 mm. The animals were subsequently tested for their rotational response to amphetamine at monthly intervals. For this purpose, each animal was placed in a hemispheric bowl 40 cm in diameter and the number of complete 360° turns was counted continuously for 2 hr following the intraperitoneal (IP) injection of d, l-amphetamine sulfate, 2.5 mg/kg, as base.

Three months after lesioning, several animals were selected who exhibited at least 500 ipsilateral turns in 2 hr following amphetamine, which is thought to act presynaptically by preferentially releasing dopamine on the intact (non-lesioned) side [14]. These animals also circled contralaterally following the IP administration of apomorphine or l-dopa, which are thought to preferentially stimulate dopamine receptors post-synaptically on the lesioned side [14]. The rotational responses of the animals to l-dopa, deprenil, l-dopa plus deprenil, clorgyline, clorgyline plus l-dopa, or saline were then determined. Deprenil or clorgyline was administered intraperitoneally at 20 mg/kg in an aqueous solution 1 hr prior to l-dopa. l-Dopa was dispersed in 0.25% methyl cellulose and administered intraperitoneally at a dose of 5 to 40 mg/kg. The dose of l-dopa used was for each individual rat on the low to middle end of its dose-response curve for l-dopa. Thus, in each animal any potentiation might be observed. Thus different rats received different doses of l-dopa. Drugs were administered in a volume of 2 ml per kg of body weight. The 6-hydroxydopamine was purchased from Regis, (Morton Grove, IL), the l-dopa was purchased from ICN Chemicals, (Cleveland, OH), the l-deprenil was a gift from Medipex (Budapest, Hungary), and the clorgyline was a gift from May and Baker Ltd. It should be mentioned that female rats are used in the rotational experiments because they grow at a slower rate than males and are thus easier to house in long-term experiments. Qualitatively similar results could be expected however, with male rats in these experiments.

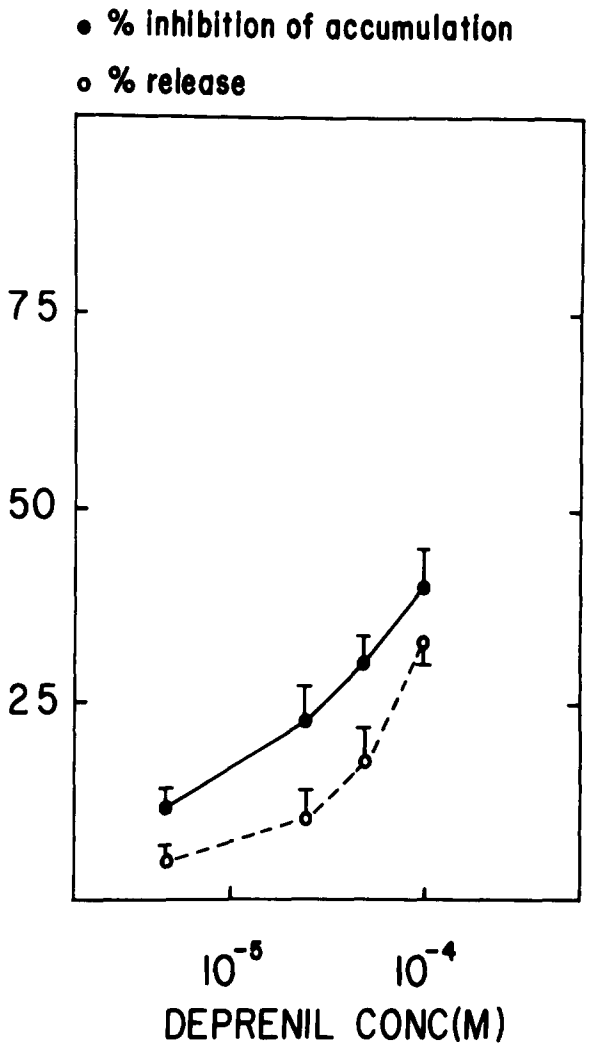

FIG. 1. The effect of l-deprenil on the accumulation or release of ^{3}H-dopamine in rat neostriatal slices. Data represent the mean±SD for three separate experiments each run in quadruplicate.

Accumulation and Release of ^{3}H-dopamine

The accumulation of ^{3}H-dopamine (^{3}H-DA, 12.0 Ci/mmole, New England Nuclear, Boston, MA) into rat brain tissue slices was carried out according to previously published techniques [7,8]. Briefly, male Sprague-Dawley rats weighing approximately 150 g were sacrificed and the neostriatum dissected out. The tissue was chopped into slices (1.0×0.2×0.2 mm) and dispersed with a magnetic stirrer into 200 volumes of a Kreb's-Ringer phosphate buffer at pH 7.4 containing 5.6 mM glucose, 1.3 mM EDTA, 1.7 mM ascorbate, and 0.08 mM pargyline [8]. Ten mg of the tissue in 2 ml of the above buffer was added to 8 ml of the buffer. Samples were then equilibrated at 37°C for 5 to 10 min, and the ^{3}H-DA (9×10^5 dpm) and the l-deprenil at the desired concentration added simultaneously. Control samples contained no deprenil. The final concentration of ^{3}H-dopamine used was 3.4 nM. The amount of radioactivity accumulated in the tissue slices was measured at 15 min as described previously, after filtration through Gooch crucibles over 2.1 cm Whatman No. 540 filter paper followed by a saline rinse [7,8].

In release studies, ^{3}H-dopamine was added to the tissue slices and accumulation done as above. After filtration and the saline rinse, the filter paper discs with adhering tissue slices were placed in 30 ml beakers containing 10 ml of the buffer. The appropriate deprenil concentration was added and the amount of radioactivity measured after a 15 min incubation at 37°C. The data were calculated as % inhibition of accumulation or % release, based on controls containing no deprenil.

RESULTS

Accumulation and Release of ^{3}H-dopamine

l-Deprenil was a weak inhibitor of ^{3}H-dopamine accumulation and a weak releasing agent for previously accumulated ^{3}H-DA in slices of rat neostriatum (Fig. 1). Note that the dose-response curves are distinctly separated. In a previous study [6], the curves for % inhibition of accumulation and %

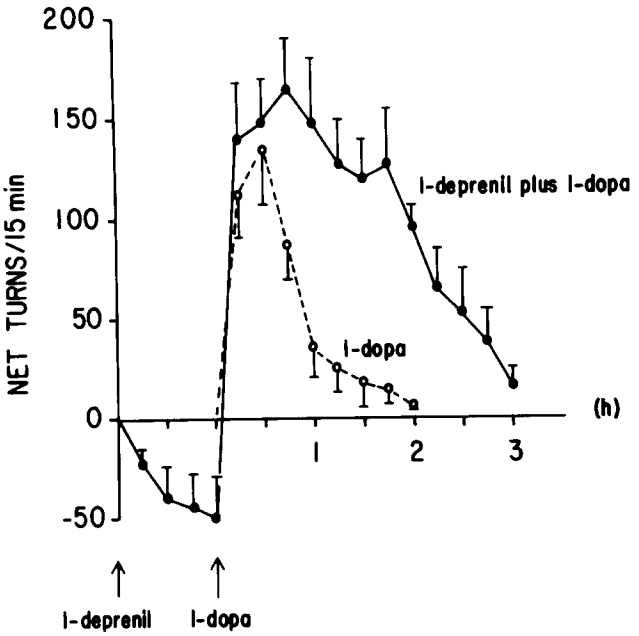

FIG. 2. The effects of l-deprenil (20 mg/kg) and l-dopa on rotational behavior in 6-hydroxydopamine lesioned rats (n=5). Two of the rats received l-dopa at 20 mg/kg and three of the rats received l-dopa at 40 mg/kg (see Method for rationale for different doses). Data represent the mean±SEM (negative numbers indicate ipsilateral turns; positive contralateral).

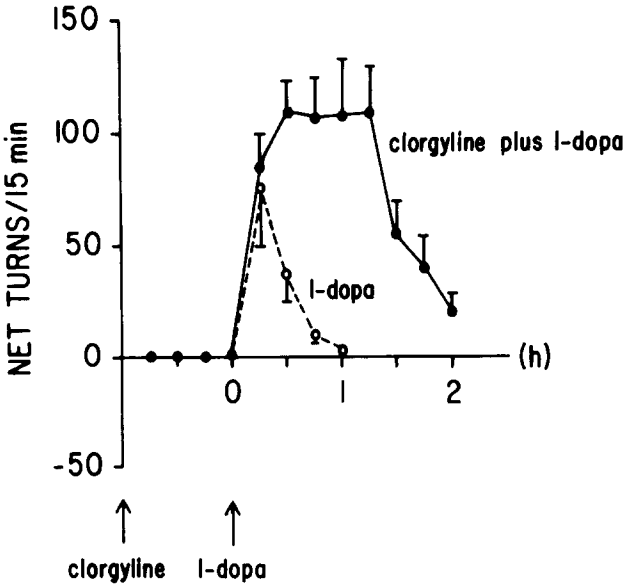

FIG. 3. The effects of clorgyline (20 mg/kg) and l-dopa on mean contralateral rotational behavior in 6-hydroxydopamine lesioned rats. (n=5) Doses of l-dopa alone in the rats were 5, 5, 10, 10 and 20 mg/kg. The animals used in these experiments were different from those used in Fig. 2. Data represent the mean turns±SEM (note that all turns were contralateral).

release for d-amphetamine were superimposable. As previously discussed [7], when this latter situation arises, the drug being studied can only be classified as a releasing agent and the observed inhibition of accumulation can be ascribed to the releasing action of the drug. Amphetamine was approximately 100 times more potent than deprenil as a releasing agent. It should be emphasized that pargyline, which was a component of the incubation medium, had no effect either on control samples or on the deprenil samples. This most likely is due to the fact that MAO inhibition has little effect during the short time periods for measurement of uptake and release.

Rotation in 6-OHDA Lesioned Rats

l-Dopa by itself causes a relatively potent contralateral rotation in rats with a unilateral 6-OHDA lesion of the substantia nigra (Fig. 2). In contrast, deprenil given alone causes a moderate ipsilateral rotation (Fig. 2). However, when l-dopa was given 1 hr after 20 mg/kg deprenil, there was a considerably greater contralateral rotation than when the same animals received l-dopa alone (Fig. 2) and, the duration of rotation was greatly increased. Three of the rats received l-dopa at 40 mg/kg, while two rats received l-dopa at 20 mg/kg. Different doses of l-dopa were used because preliminary studies showed the chosen doses to give rotation on the low to intermediate part of the dose-response curve. There was a somewhat variable response to l-dopa alone, but deprenil pre-treatment brought about a large potentiation of the dopa response in each animal; ratios of contralateral rotation to l-dopa with and without deprenil ranged from 1.8 to 4.5. In other experiments d-amphetamine failed to potentiate and in fact diminished somewhat the rotational response to l-dopa.

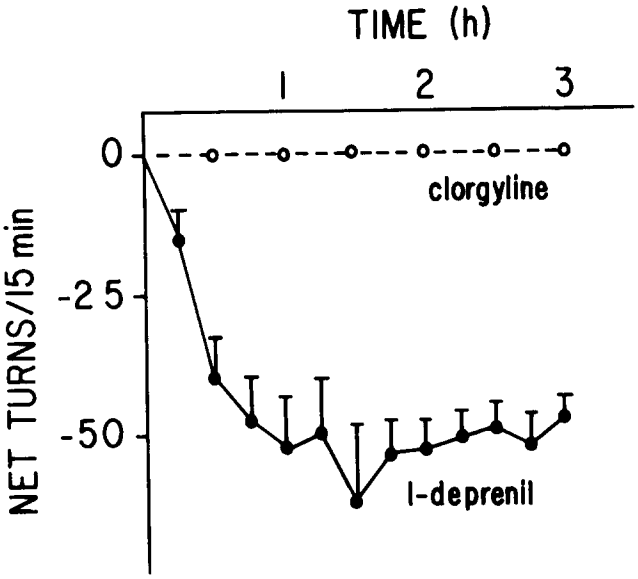

FIG. 4. Rotational behavior after l-deprenil or clorgyline at 20 mg/kg. Results are the mean values±SEM for 5 rats. Note that the turns are ipsilateral (left) for deprenil.

Amphetamine (0.5 mg/kg in saline) or saline alone was given to rats. After 1 hr, l-dopa was given and rotation was measured for 2 hr. It should be emphasized that the rats were still rotating ipsilaterally to the dose of d-amphetamine at the time they received l-dopa. The animals receiving saline followed by l-dopa had 409±74 net contralateral turns (n=3)

while the same animals receiving d-amphetamine followed by l-dopa had 155±61 net contralateral turns (n=3). Ratios of the contralateral rotation to l-dopa with amphetamine and without ranged from 0.2 to 0.7 for the individual rats.

Clorgyline like deprenil, also greatly potentiated the rotational response brought about by l-dopa (Fig. 3). But clorgyline alone at 20 mg/kg caused no rotation (Fig. 4). In contrast, rats rotated for at least 3 hr following 20 mg/kg of l-deprenil (Fig. 4). In these studies, experiments were first done with clorgyline in all rats. One week later, experiments with deprenil were done. It should be emphasized that similar rotational responses to deprenil were obtained in separate experiments in rats that had not received a prior clorgyline injection. Rats given clorgyline one week after deprenil similarly failed to rotate.

DISCUSSION

Analysis of Accumulation and Release Data: Correlations with Rotation

l-Deprenil was quite weak as an uptake inhibitor or as a releasing agent for previously accumulated ^{3}H-DA in rat neostriatal slices. It is generally accepted that drugs which increase dopaminergic activity by dopamine release or by blockade of dopamine uptake cause ipsilateral rotation in rats with a 6-OHDA lesion of the nigrostriatal pathway [3,14]. Deprenil by itself causes a relatively weak ipsilateral rotation in the 6-OHDA lesioned rat. This ipsilateral rotation might conceivably be due either to the uptake inhibition, or the dopamine releasing action of deprenil itself [3] or amphetamine-like metabolites of deprenil. One might postulate that deprenil would inhibit MAO activity preferentially on the intact (non-lesioned) neostriatum which also could explain the ipsilateral rotation. However, clorgyline which is a well-known and widely used inhibitor of MAO and which is not known to be a releasing agent or uptake inhibitor for DA, by itself caused no rotation in the same animals (see Fig. 4). It is thus unlikely that the rotational behavior induced by deprenil reflected MAO inhibition. Rather it appears most probable that release of DA (or DA uptake inhibition) brought about by deprenil or its metabolites was responsible for the observed rotation. The possibility that increased accumulation of phenylethylamine on the non-lesioned side played a role [2] cannot be excluded.

When l-dopa was given to deprenil pretreated rats, there was a substantially greater contralateral rotation than in the same rats given l-dopa alone. It seems unlikely that the potentiation of l-dopa induced rotation was due to either the uptake inhibition or to the releasing capacity of deprenil or its metabolites because, (1) uptake inhibitors cause ipsilateral whereas l-dopa causes contralateral rotation, and (2) the releasing agent amphetamine, which also causes ipsilateral rotation, did not enhance the l-dopa response. The potentiation of l-dopa induced rotation by deprenil is most likely due to the MAO inhibitory properties of deprenil. The potentiation of rotation by clorgyline, which is not known to have any releasing action but is a potent MAO inhibitor, lends credence to this concept.

General Considerations

Clinical observations to date have indicated that deprenil potentiates the effect of l-dopa in Parkinson patients and permits the dosage of l-dopa to be reduced by 30 to 50% [1,11]. Evaluation of the effect of deprenil in patients with short duration responses to l-dopa have shown that the on-off effect is effectively diminished and the duration of the response to individual doses of l-dopa is prolonged [16].

The present data document the potentiation by deprenil of the behavioral effect of l-dopa in unilaterally nigral-lesioned rats. The data of the present study, taken at face value, suggest that other MAO inhibitors might, like deprenil, be useful in combatting parkinsonism. However, the fact that dopamine is handled primarily by MAO-B in humans (as opposed to both forms of MAO in the rat) combined with the fact that several type A or mixed MAO inhibitors appear to cause unpleasant or even dangerous side effects when combined with l-dopa, would preclude the usage of MAO-A or mixed inhibitors in the Parkinson patient. However, other type B MAO inhibitors that would not cause serious side effects when combined with dopa, might potentially be of therapeutic value.

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