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Conditioned Place Preference Induced by a Combination of L-Dopa and a COMT Inhibitor, Entacapone, in Rats

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KATAJAMÄKI, J., A. HONKANEN, T. P. PIEPPONEN, I.-B. LINDÉN, A. ZHARKOVSKY AND L. AHTEE. *Conditioned place preference induced by a combination of L-dopa and a COMT inhibitor, entacapone, in rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 23–26, 1998.—The interaction of dopamine (DA) precursor L-dopa and catechol-O-methyltransferase (COMT) inhibitor, entacapone, was examined in rats using conditioned place preference (CPP) paradigm to assess reinforcement, and by measuring DA metabolism in the striatum and the limbic forebrain. Neither L-dopa (100 mg/kg IP) nor entacapone (30 mg/kg IP) alone induced CPP, but in combination they induced significant CPP. Entacapone alone had no effect on limbic or striatal DA concentrations, while it reduced the concentrations of the COMT products 3-methoxytyramine (3-MT), a metabolite reflecting DA release, and homovanillic acid (HVA) in both brain areas. L-dopa elevated limbic but not striatal 3-MT. L-dopa also slightly elevated limbic DA but had no effect on striatal DA concentration. L-Dopa–induced increase of 3-MT was attenuated by entacapone. Our results show for the first time that L-dopa is able to produce CPP in intact animals. This effect may be related to the findings that L-dopa increases synaptic DA concentrations in the limbic areas, and entacapone may enhance this elevation as it prevents the synaptic metabolism of DA. © 1998 Elsevier Science Inc.

L-Dopa Entacapone Conditioned place preference Dopamine 3-Methoxytyramine Reinforcement

THE mesolimbic dopamine (DA) has been suggested to be critically involved in the reinforcing effects of drugs of abuse (11). For example, psychostimulants such as amphetamine, which releases DA into the synaptic cleft, and cocaine, which increases synaptic DA by blocking its uptake, are powerful reinforcers. Furthermore, rats have been shown to self-administer DA directly into the nucleus accumbens (5).

The DA precursor L-dopa is used to increase cerebral DA concentrations and thereby to alleviate the signs and symptoms of Parkinson's disease. L-dopa penetrates the blood–brain barrier poorly, and less than 1% of an orally administered dose reaches the brain. Therefore, peripheral decarboxylase inhibitors are used to allow a greater proportion of L-dopa to reach the brain. Recently, selective catechol-O-methyl transferase (COMT) inhibitors, such as entacapone, have been developed to further improve the bioavailability of L-dopa in the brain (2,13,18).

In clinical studies, the antiparkinsonian activity of L-dopa combined with a decarboxylase inhibitor was prolonged and enhanced by entacapone (19,22). Furthermore, several patients reported improved sleep, energy levels and their sense of well-being while receiving entacapone (19). This clinical observation could be related to possible reinforcing effects of the combination, particularly because there are some reports of the abuse of L-dopa (16,20). However, there is no evidence for the reinforcing effect of L-dopa in experimental animals. Thus, we have studied the effects of L-dopa combined with entacapone using the conditioned place preference (CPP) paradigm in rats. This technique assesses drug reward by measuring the association developed between certain neutral envi-

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ronmental stimuli and the primary drug effect (23). Therefore, to investigate the effectiveness of L-dopa and entacapone to induce changes in cerebral DA turnover we measured the concentrations of DA and its metabolites 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the striatum and the limbic forebrain of rat after acute administration of the combination. Indeed, these biochemical experiments suggest that the drug combination selectively elevates the synaptic DA concentration in the terminal mesolimbic areas.

METHOD

Animals

Male Wistar rats (250–350 g) were housed in groups of four to six under 12 L:12 D cycle (lights on at 0500 h). Water and standard laboratory food were available ad lib except during conditioning and test sessions.

Drugs

Entacapone (N,N-diethyl-2-cyano-3-(3,4,-dihydroxy-5-nitrophenyl) acrylamide; Orion Pharma, Espoo, Finland) was suspended in a 20% solution (w/v) of hydroxypropyl-β-cyclodextrin (Aldrich-Chemie, Steinheim, Germany) prepared in distilled water. Levodopa methyl ester hydrochloride (Sigma Chemical Company, MO), a prodrug of L-dopa, was dissolved in distilled water. All treatments were given intraperitoneally (IP, 0.2 ml/100 g) and doses refer to the acid or base form. Control rats received either saline or 20% solution of hydroxypropyl-β-cyclodextrin (vehicle). Reagents used in HPLC assays were p.a. grade and purchased from E. Merck (Darmstadt, Germany) except dibutylamine (DBH, UK) and octanesulphonic acid (Fluka Chemie, Switzerland).

Place Conditioning

Place conditioning was conducted as previously described (10). The apparatus consisted of two square-base compartments (h 40 × 30 × 30 cm) covered with a transparent ceiling, one with white and the other with dark gray walls and floor. For 3 days rats had free access to both compartments for 15 min (900 s) each day. On day 3, the time spent by the rats in each compartment was recorded and these values served as a baseline. Conditioning was conducted for 4 days and included two sessions each day; one with drug treatments and one with vehicle. The rats were given entacapone 30 mg/kg or vehicle 15 min before L-dopa 50 or 100 mg/kg or 0.9% saline (controls) immediately before placing them in the nonpreferred compartment for 60 min. After an interval of 4 h all the rats received vehicle and saline as described above and were placed in the preferred compartment for 60 min. The order of drug and vehicle presentation was balanced across treatment groups. On day 8, the rats were allowed to freely choose their preferred compartment in the apparatus for 15 min and the time spent in each compartment was recorded.

Dopamine Metabolism

In the biochemical experiments the rats were given entacapone 30 mg/kg or vehicle (pretreatment) 15 min before L-dopa 100 mg/kg or saline (treatment). The rats were sacrificed 60 min after the last injection by a high-intensity microwave irradiation focused on the head using the NJE-10 kW microwave irradiator (New Japan Radio Inc., Japan) set at 7 kW and 1.4 s. The brains were removed from the skull and placed in a stainless steel brain mold. The caudate-putamen (striatum) and the limbic forebrain (containing the nucleus accumbens, olfactory tubercle, and amygdala) were dissected as described previously (8). The brain tissues were stored at −80°C until DA and its metabolites were assayed by using HPLC with electrochemical detection (8).

Statistics

The results of the CPP experiments were analyzed using analysis of covariance (ANCOVA), and when appropriate, Student's *t*-test. The results of the biochemical analyses were tested by two-way analysis of variance (ANOVA), and when appropriate, Tukey compromise post hoc test.

RESULTS

In a pilot experiment the effects of 50 and 100 mg/kg doses of L-dopa administered 15 min after entacapone 30 mg/kg were studied. The dose of 50 mg/kg of L-dopa with entacapone had no place-conditioning effect, but the dose of 100 mg/kg of L-dopa with entacapone induced significant CPP compared with the control, $F(1, 15) = 12.2$, $p < 0.01$ (data not shown). In the CPP experiments there is often a "step-up" dose–effect relationship where one dose of a drug does not induce CPP but the next higher dose produces a positive effect that is also the maximal effect (23). Therefore, we conducted further experiments with the dose of 100 mg/kg of L-dopa.

Entacapone (30 mg/kg) or L-dopa (100 mg/kg) alone had no effect on the place preference of the animals (Fig. 1). However, in combination, these treatments induced significant CPP, ANCOVA $F(1, 46) = 8.5$, $p < 0.01$.

L-dopa (100 mg/kg, Table 1) had no effect on striatal DA but it tended to increase the concentration of DA in the limbic forebrain, $F(1, 21) = 5.9$, $p < 0.05$. However, there were no significant differences in post hoc comparisons. L-dopa profoundly elevated both limbic and striatal DOPAC and HVA concentrations, $F(1, 21) > 297$, $p < 0.001$. The striatal 3-MT concentration was not altered by L-dopa, $F(1, 21) = 1,9$, $p = 0.18$. However, limbic 3-MT concentration was increased

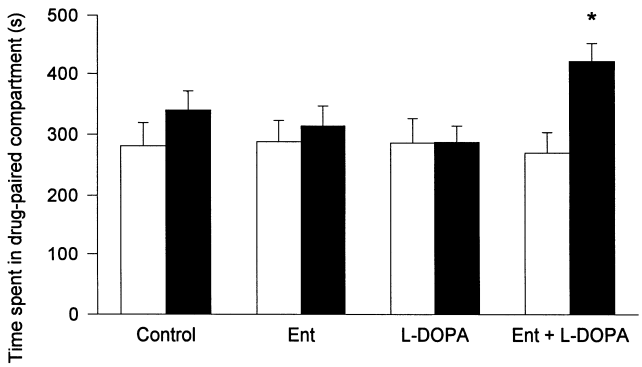

FIG. 1. Effects of entacapone (Ent, 30 mg/kg IP), L-dopa (100 mg/kg IP) and a combination of them on conditioned place preference. The rats were given entacapone or vehicle 15 min before L-dopa or saline and immediately placed in the place preference box for conditioning on 4 consecutive days. White columns represent the time spent in the less preferred compartment in the preconditioning session and filled columns the time spent in the same compartment in the postconditioning session. The data are expressed as means ± SEM, $n = 12$. *$p < 0.05$ compared with control (Student's *t*-test).

TABLE 1

EFFECTS OF ENTACAPONE, L-DOPA AND THEIR COMBINATION ON THE CONCENTRATIONS OF DOPAMINE AND ITS METABOLITES IN RAT BRAIN

Treatment	DA (μg/g)	3-MT (ng/g)	DOPAC (μg/g)	HV A (μg/g)
Striatum				
Control	12.2 ± 1.2	25.3 ± 1.1	1.1 ± 0.1	0.8 ± 0.1
L-dopa	12.6 ± 1.2	21.9 ± 0.8	8.9 ± 0.4†	5.5 ± 0.2†
Entacapone	10.9 ± 0.9	12.0 ± 2.0†‡	1.6 ± 0.1	0.5 ± 0.1
Entacapone + L-dopa	11.0 ± 1.1	12.0 ± 0.7†‡	14.6 ± 0.2†‡	3.6 ± 0.4†‡
Limbic forebrain				
Control	2.4 ± 0.2	3.6 ± 0.3	0.25 ± 0.002	0.13 ± 0.006
L-dopa	2.9 ± 0.1	11.4 ± 0.3†	7.1 ± 0.6†	3.2 ± 0.1†
Entacapone	2.6 ± 0.1	2.3 ± 0.3*‡	0.36 ± 0.04	0.09 ± 0.02
Entacapone + L-dopa	2.8 ± 0.1	5.9 ± 0.2†‡	10.0 ± 0.6†‡	2.5 ± 0.1†‡

Entacapone (30 mg/kg IP) or vehicle was given 15 min before L-dopa (100 mg/kg IP) or saline and the rats were sacrificed 60 min after L-dopa. The data are means ± SEM, $n = 6–7$.

*$p < 0.05$; † $p < 0.01$ as compared with control.

‡ $p < 0.01$ compared with L-dopa (Tukey compromise post hoc test).

several fold, $F(1, 21) = 430, p < 0.001$. Entacapone (30 mg/kg) had no effect on the concentrations of limbic and striatal DA but increased DOPAC, $F(1, 21) > 12.2$, $p < 0.01$, and decreased HVA, $F(1, 21) > 14.2, p < 0.01$, and 3-MT, $F(1, 21) > 84.1, p < 0.001$, concentrations in these brain areas. A combination of L-dopa and entacapone did not alter DA concentrations in either brain area studied but increased limbic and striatal DOPAC more than L-dopa alone [pretreatment × treatment interaction: $F(1, 21) = 18.5, p < 0.001$; $F(1, 21) = 10.5$, $p < 0.01$, respectively]. Entacapone pretreatment reduced the L-dopa–induced elevation of the limbic and striatal HVA concentration, $F(1, 21) > 10.9, p < 0.01$). Entacapone also attenuated the L-dopa–induced increase of limbic 3-MT [interaction: $F(1, 21) = 61.8, p < 0.001$].

DISCUSSION

The combination of entacapone 30 mg/kg and L-dopa 100 mg/kg induced CPP, suggesting that this treatment is reinforcing in rats. The most obvious explanation for this effect is that the combination activates the limbic dopaminergic mechanisms mediating reinforcement (11). Indeed, parkinsonian patients have abused levodopa in escalating doses (16,20). However, we have not found any previous experimental evidence to indicate a reinforcing action of L-dopa. In our experiment neither L-dopa nor entacapone alone induced CPP. In addition, when entacapone was administered with a smaller dose (50 mg/kg) of L-dopa, no CPP occurred. Because there was no peripheral decarboxylase inhibitor included in our study, it is possible that the smaller dose of L-dopa failed to elevate cerebral DA concentrations enough due to peripheral metabolism.

COMT is expressed in two forms, membrane bound (MB)-COMT and soluble (S)-COMT (21). COMT has been suggested to inactivate DA released into the synaptic cleft by metabolising it to 3-MT (21,24). Thus, entacapone could increase especially the synaptic concentration of DA by inhibiting its conversion to 3-MT in the L-dopa–treated rats. Entacapone penetrates the blood–brain barrier poorly (13), but in large doses it is able to inhibit COMT in brain. This can be seen clearly from our finding that entacapone reduced concentrations of 3-MT in both brain areas studied.

L-dopa (100 mg/kg) alone elevated limbic 3-MT concentration over 200%, suggesting that it increased synaptic dopamine, too. Therefore, it is amazing that L-dopa did not induce CPP. Because exogenous L-dopa is partially converted to DA in nondopaminergic cells, where either MB-COMT or S-COMT may be expressed (7,14,17), it is possible that some of the DA is methylated before it is released into the synaptic cleft. Thus, L-dopa–induced increase in limbic 3-MT concentration does not necessarily reflect only the change in synaptic DA level but also DA formation and metabolism in the nondopaminergic cells (14). Entacapone could further increase the synaptic DA concentration to a level that stimulates DA receptors mediating reward by inhibiting the methylation of DA in nondopaminergic cells. This hypothesis is supported by the findings that COMT inhibitors further elevate the striatal extracellular DA in rats treated with L-dopa + dopa-decarboxylase inhibitor (2,9,15).

The DA concentrations were only slightly elevated in the limbic forebrain, and no changes were found in the striatum by the treatments used. This is most probably due to rapid catabolism of newly synthetized DA after L-dopa treatment. However, coadministration of L-dopa with dopadecarboxylase inhibitors has been reported to elevate striatal DA concentrations (6,7,18).

In contrast to its effect in the limbic forebrain, L-dopa did not increase 3-MT concentration in the striatum but tended to decrease it. This is in accordance with previous reports showing that L-dopa, combined with peripheral dopadecarboxylase inhibitor, transiently decreases DA release (1,6). This was suggested to result from an activation of DA autoreceptors controlling DA release. Our results thus suggest that DA release is controlled by feedback mechanism more efficiently in the striatum than in the limbic areas. However, Bunney et al. (4) did not find any differences between the actions of L-dopa on the firing rate of DA neurons in the ventral tegmental area and the substantia nigra. Alternatively, the rates of decarboxylation of L-dopa and methylation of DA differ in the striatum and the limbic forebrain. Indeed, it is well-documented that there are differences in the synthesis and metabolism of DA between striatal and limbic brain regions (3,8,12).

In conclusion, our results show that exogenous L-dopa not alone, but when given together with entacapone is able to produce reinforcing effects in rats. This is the first experimental evidence that L-dopa is able to produce reinforcement in rats. This effect may be related to the findings that L-dopa in-

creases synaptic DA concentrations in the limbic areas, and entacapone may enhance this elevation as it prevents synaptic metabolism of DA. Our results do not prove, of course, that the sense of well-being in parkinsonian patients receiving entacapone results from the reinforcing action of this combination. It should especially be remembered that in the clinical dose range entacapone penetrates the blood–brain barrier poorly and a relatively large dose was used in our experiments. Furthermore, our experiments were conducted in intact animals. The action of L-dopa on DA transmission in a parkinsonian brain may dramatically differ from that in a normal brain (1), so even a minor COMT inhibition combined with levodopa may produce potent effects in the parkinsonian brain.

REFERENCES

1. Abercrombie, E. D.; Bonatz, A. E.; Zigmond, M. J.: Effects of L-DOPA on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. Brain Res. 525:36–44; 1990.
2. Acquas, E.; Carboni, E.; de Ree, R. H. A.; Da Prada, M.; Di Chiara, G.: Extracellular concentrations of dopamine and metabolites in the rat caudate after oral administration of a novel catechol-O-methyltransferase inhibitor Ro 40-7592. J. Neurochem. 59:326–330; 1992.
3. Andén, N.-E.; Grabowska-Andén, M.; Lindgren, S.; Thornström, U.: Synthesis rate of dopamine: Difference between corpus striatum and limbic system as a possible explanation of variations in reactions to drugs. Naunyn Schmiedebergs Arch. Pharmacol. 323:193–198; 1983.
4. Bunney, B. S.; Aghajanian, G. K.; Roth, R. H.: Comparison of effects of L-dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurones. Nature 245:123–125; 1973.
5. Dworkin, S. I.; Goeders, N. E.; Smith, J. E.: The reinforcing and rate effects of intracranial dopamine administration. NIDA Res. Monogr. 67:242–248; 1986.
6. El Gemayel, G.; Trouvin, J. H.; Prioux-Guyonneau, M.; Jacquot, C.; Cohen, Y.: Dopaminergic metabolism in various rat brain areas after L-dopa loading. J. Pharm. Pharmacol. 38:691–694; 1986.
7. Hefti, F.; Melamed, E.; Wurtman, R. J.: The site of dopamine formation in rat striatum after L-dopa administration. J. Pharmacol. Exp. Ther. 217:189–197; 1981.
8. Honkanen, A.; Piepponen, T. P.; Ahtee, L.: Morphine-stimulated metabolism of striatal and limbic dopamine is dissimilarly sensitized in rats upon withdrawal from chronic morphine treatment. Neurosci. Lett. 180:199–222; 1994.
9. Kaakkola, S.; Wurtman, R. J.: Effects of COMT inhibitors on striatal dopamine metabolism: A microdialysis study. Brain Res. 587:241–249; 1992.
10. Kivastik, T.; Vuorikallas, K.; Piepponen, T. P.; Zharkovsky, A.; Ahtee, L.: Morphine and cocaine-induced conditioned place preference: Effects quinpirole and preclamol. Pharmacol. Biochem. Behav. 54:371–375; 1996.
11. Koob, G. F.: Drugs of abuse: Anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13:177–184; 1992.
12. Kuczcenski, R.; Segal, D. S.: Differential effects of amphetamine and dopamine uptake blockers (cocaine, nomifensine) on caudate and accumbens dialysate dopamine and 3-methoxytyramine. J. Pharmacol. Exp. Ther. 262:1085–1094; 1992.
13. Männistö, P. T.; Ulmanen, I.; Lundström, K.; Taskinen, J.; Tenhunen, J.; Tilgman, C.; Kaakkola, S.: Characteristics of catechol-O-methyltransferase (COMT) and properties of selective COMT inhibitors. Prog. Drug. Res. 39:291–350; 1992.
14. Melamed, E.; Hefti, F.; Wurtman, R. J.: Nonaminergic striatal neurons convert exogenous L-dopa to dopamine in parkinsonism. Ann. Neurol. 8:558–563; 1980.
15. Napolitano, A.; Zürcher, G.; Da Prada, M.: Effects of tolcapone, a novel catechol-O-methyltransferase inhibitor, on striatal metabolism of L-dopa and dopamine in rats. Eur. J. Pharmacol. 273:215–221; 1995.
16. Nausieda, P. A.: Sinemet "abusers". Clin. Neuropharmacol. 8:318–327; 1985.
17. Ng, L. K. Y.; Chase, T. N.; Coldburn, R. W.; Kopin, I. J.: L-dopa in parkinsonism. Neurology 22:688–695; 1972.
18. Nissinen, E.; Lindén, I.-B.; Schultz, E.; Pohto, P.: Biochemical and pharmacological properties of peripherally acting catechol-O-methyltransferase inhibitor entacapone. Naunyn Schmiedebergs Arch. Pharmacol. 346:262–266; 1992.
19. Nutt, J. G.; Woodward, W. R.; Beckner, R. M.; Stone, C. K.; Berggen, K.; Carter, J. H.; Gancher, S. T.; Harmmerstad, J. P.; Gordin, A.: Effect of peripheral catechol-O-methyltransferase (COMT) inhibition on the pharmacokinetics and pharmacodynamics of levodopa in parkinsonian patients. Neurology 44:913–919; 1994.
20. Priebe, S.: Levodopa dependence: A case report. Pharmacopsychiatry 17:109–110; 1984.
21. Roth., J. A.: Membrane-bound catechol-O-methyltransferase: A reevaluation of its role in the O-methylation of catecholamine neurotransmitters. Rev. Physiol. Biochem. Pharmacol. 120:1–29; 1992.
22. Ruottinen, H. M.; Rinne, U. K.: Entacapone prolongs levodopa response in a one month double blind study in parkinsonian patients with levodopa related fluctuations. J. Neurol. Neurosurg. Psychiatry 60:36–40; 1996.
23. Schechter, M. D.; Calcagnetti, D. J.: Trends in place preference conditioning with a cross-indexed bibliography; 1957–1991. Neurosci. Biobehav. Rev. 17:21–41; 1993.
24. Wood, P. L.; Altar, C. A.: Dopamine release in vivo from nigrostriatal, mesolimbic, and mesocortical neurons: Utility of 3-methoxytyramine measurements. Pharmacol. Rev. 40:163–187; 1988.