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Short communication

Beneficial effects of co-administration of catechol-*O*-methyltransferase inhibitors and L-dihydroxyphenylalanine in rat models of depression

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Abstract

The administration of catechol-*O*-methyltransferase inhibitors alone changed neither the behavior of the rats in two animal models of depression, the forced swimming test (entacapone and tolcapone) or in the learned helplessness paradigm (tolcapone), nor the locomotor activity. L-Dihydroxyphenylalanine (L-DOPA) and carbidopa treatment as such decreased motility but did not improve the behavior in the antidepressant tests. Co-administration of catechol-*O*-methyltransferase inhibitors and L-DOPA/carbidopa increased the performance of rats in both tests without increasing locomotor activity. Catechol-*O*-methyltransferase inhibitors could be beneficial as adjunct drugs of L-DOPA not only in Parkinson's disease but also in the coincident depressive illness.

Keywords: Catecholamine, depression; Depression, animal model; Catechol-*O*-methyltransferase, inhibitor

1. Introduction

The implication of the dysfunction of serotoninergic and adrenergic systems in the pathophysiology of depression is well established. However, it seems that a deficit in dopamine function is present in depression as well. Reduced dopaminergic neurotransmission contributes to the pathophysiology of retarded depression (Rampello et al., 1991) and may play a part in suicidal behavior (Roy et al., 1992). Approximately one-third of the patients having Parkinson's disease are suffering from depression (Cummings, 1992).

The results of animal experiments support the dopamine hypothesis of depression. The decreased performance of animals in the learned helplessness paradigm was related to the reduced levels of dopamine in the nucleus accumbens and nucleus caudatus (Willner, 1985). Antidepressive drugs affect the dopaminergic neurotransmission. Facilitation of dopaminergic function has been observed after repeated administration of antidepressive drugs (Plaznik and Kostowski, 1987). During chronic antidepressant treatment hypersensitivity appears at postsynaptic dopamine receptors, whereas subsensitivity develops at presynaptic dopamine receptors (Maj, 1990).

Entacapone and tolcapone are potent and selective inhibitors of catechol-*O*-methyltransferase. Owing to its slight brain penetration, entacapone is active in peripheral tissues while tolcapone is inhibiting catechol-*O*-methyltransferase also in the brain (Kaakkola et al., 1994; Männistö, 1994). Since little is known about the behavioural effects of these compounds, which may increase both dopaminergic and noradrenergic neurotransmission in the brain, we have assessed the action of catechol-*O*-methyltransferase inhibitors (alone and in combination with L-dihydroxyphenylalanine (L-DOPA) and carbidopa) in two animal models of depression.

2. Materials and methods

2.1. Animals

Male Wistar rats (Department of Physiology, University of Helsinki, Finland), weighing 180–250 g, were

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used in experiments. The animals were maintained on food (animal pellets) and tap water ad libitum. Rats were kept in a temperature-controlled dark room (22 ± 1°C) having an artificial 12 h light-dark cycle (lights on at 7:00 h).

2.2. Forced swimming test

The procedure was a modification of that reported by Porsolt and coworkers (1978). Sessions were started by placing individual rats in rectangular glass water containers (20 × 22 × 50 cm) containing 30 cm of water (27–28°C). Two subsequent sessions were performed. The first 15-min training session was followed 24 h later by a 5-min test session. After training sessions, the rats were towel-dried and housed singly until testing on the next day. On the second day, the floating time of the animal was recorded by the observer who was not aware of the treatments. A rat was judged to be floating when it was not making any other movements but those necessary to keep its head above the water.

2.3. Learned helplessness test

The test method was a minor modification of that described by Giral and coworkers (1988). Preconditioning with inescapable electric foot shock was given individually to each rat in the rectangular Plexiglas chamber (24 × 21 × 21 cm). The grid floor was made of stainless-steel rods spaced 1.5 cm apart. A constant-current shocker delivered 60 scrambled, inescapable shocks (15-s duration, 0.8 mA, every 1 min) to the grid floor. Control rats were placed in the identical chambers for 1 h, but shock was not given. All preconditioning trials were performed on day 1. Conditioned avoidance sessions were performed on day 5. Animals were placed individually into the Plexiglas shuttle-box (48 × 21 × 21 cm) having a grid floor, made of stainless-steel rods spaced 1.5 cm apart. The shuttle-box was divided into two equal chambers by a stainless-steel wall which had an open door (diameter 8 cm) between them. The animals were allowed to habituate to the environment for 5 min and then subjected to 30 trials. The intertrial interval was 30 s. During the first 3 s of each trial, a light signal was presented. During this period the rats could avoid shocks by moving into the other compartment of the box. If an avoidance response did not occur, a 3-s electric foot shock (0.8 mA) period was presented. Failing to escape during the shock period was recorded.

2.4. Locomotor activity

After administration of drugs at 24, 5 and 1 h before the test, the rats were kept in their home cages. The apparatus was a square open field arena (Kungsbacka Mät- & Reglerteknik, Sollentuna, Sweden; 68 × 68 × 35 cm) equipped with two rows of photocells, sensitive to infrared light. The horizontal locomotor activity and the frequency of rearing were recorded in 5 min periods for a total time of 30 min.

2.5. Drugs

Entacapone (OR-611; (*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitro-phenyl)propenamide) and tolcapone (Ro 40-7592, 4'-methyl-3,4-dihydroxy-5-nitrobenzophenone) were synthesized by Ms. Aino Pippuri, M.Sci. (Orion-Farmos, Research Center, Espoo, Finland). Their purity was higher than 99% based on nuclear magnetic resonance and infrared spectra. Entacapone and tolcapone were homogenized in saline with the help of few drops of Tween-85. L-DOPA (L-dihydroxyphenylalanine methylester hydrochloride; Sigma Chemical Co., St. Louis, MO, USA) and desipramine hydrochloride (Research Biochemicals International, Natick, MA, USA) were diluted in saline, carbidopa (Orion-Farmos) in 0.5% carboxymethylcellulose solution in saline. Doses refer to respective acids and bases.

2.6. Treatment

All drugs were administered intraperitoneally (i.p.). When the combination of catechol-*O*-methyltransferase inhibitors with L-DOPA plus carbidopa was used, enzyme inhibitors were administered 15 min before L-DOPA. Time count started from L-DOPA injection. In the forced swimming test vehicle or the drugs were administered 24, 5 and 1 h before the test session on the second day (the first injection was made 5 min after the training session). In the learned helplessness test rats were randomly treated according to one of the following protocols: the control animals, which received no shock, were given vehicle; animals with inescapable shock were injected daily for 5 consecutive days with vehicle or test compound. The first injection was given 6 h after shock on day 1, and then twice per day, one half of the daily dose in the morning and the second half dose in the afternoon. On the last day, L-DOPA was administered 30 min (and enzyme inhibitors 45 min) before the shuttle-box session.

2.7. Statistics

The mean values ± S.E.M.s are presented in the figure and table. One-way analysis of variance followed by Duncan's multiple range test was used for evaluation of data.

3. Results

3.1. Forced swimming test

Desipramine (20 and 30 mg/kg) significantly reduced floating time compared to vehicle-treated rats (Table 1). Neither entacapone (30 and 50 mg/kg) nor tolcapone (10–50 mg/kg), given alone, had influence on the floating time (Table 1). A combination of L-DOPA (10–50 mg/kg) with carbidopa (50 mg/kg), without catechol-*O*-methyltransferase inhibitors, did not affect the behavior of rats in forced swimming test (Table 1, Fig. 1A).

Co-administration of catechol-*O*-methyltransferase inhibitors (3 or 10 mg/kg) with L-DOPA and carbidopa shortened the floating time of the rats when the highest dose of L-DOPA (50 mg/kg) was used (Fig. 1A).

3.2. Learned helplessness

Desipramine (10 mg/kg, twice daily) significantly reversed the helplessness of rats during the conditioned avoidance training session making the rats to escape the shocks (escape failures: vehicle control, 20.1 ± 2.7, mean ± S.E.M.; desipramine, 10.6 ± 2.8, $P < 0.01$). None of the tested doses of tolcapone (3, 10 or 30 mg/kg, twice daily) affected the number of escape failures of helpless rats (vehicle control: 17.6 ± 2.2; 3 mg/kg, 20.6 ± 2.2; 10 mg/kg, 18.4 ± 2.9; 30 mg/kg, 16.4 ± 2.6; $n = 8–10$). The same was true with L-DOPA (10 or 50 mg/kg, twice daily) plus carbidopa (50 mg/kg, twice daily), without tolcapone (vehicle control, 17.1 ± 4.0; 10 mg/kg, 17.1 ± 2.4; 50 mg/kg, 18.1 ± 3.5; $n = 8–10$).

Tolcapone, L-DOPA and carbidopa reduced the escape failures significantly when the highest doses (L-DOPA 50 mg/kg, carbidopa 50 mg/kg and tolcapone 10 mg/kg, twice daily) were co-administered (Fig. 1B). The other combinations were ineffective (not shown). It is worthy to note that one half of the rats in the group treated with tolcapone (10 mg/kg, twice

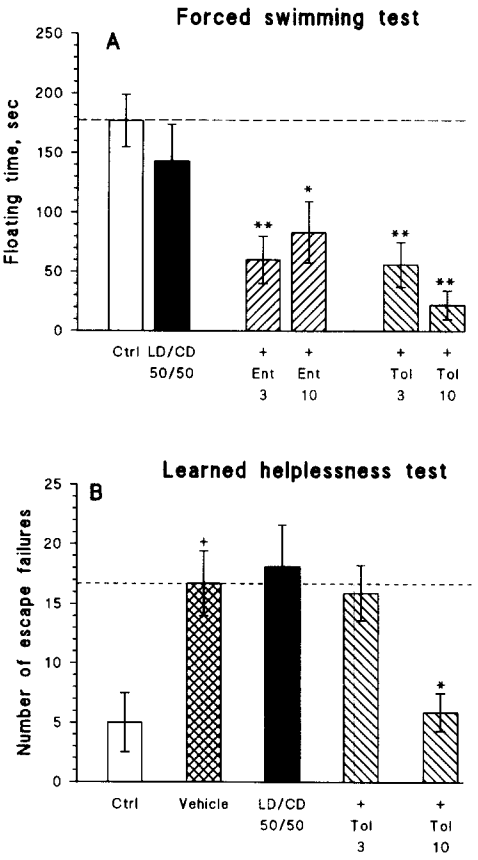

Fig. 1. (A) Effects of L-DOPA/carbidopa (LD/CD; 50/50 mg/kg) alone or with entacapone (Ent, 3 or 10 mg/kg) and tolcapone (Tol, 3 or 10 mg/kg) on the floating time in the forced swimming test in rats. Control rats (Ctrl) were given respective vehicles instead of drugs. The swimming time was 5 min. Mean ± S.E.M., $n = 9–20$. Statistics: $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (Ctrl) group. (B) Effect of vehicle, L-DOPA/carbidopa (LD/CD, 50/50 mg/kg) alone or with tolcapone (Tol, 6 or 30 mg/kg per day) on the escape failures in the learned helplessness paradigm in rats. Each rat was subjected to 30 trials. Mean ± S.E.M., $n = 8$. Statistics: $^{+}P < 0.05$, $^{++}P < 0.01$ versus control (Ctrl) rats which were not made helpless (efficacy of shocks); $^{*}P < 0.05$ versus helpless rats given only the corresponding vehicles (efficacy of tolcapone).

Table 1
Effect of desipramine, entacapone, tolcapone and L-DOPA/carbidopa combination on the floating time in the forced swimming test in male rats

	Drug-treated		Corresponding control	
Drug and dose	Floating time, s	*n*	Floating time, s	*n*
Desipramine				
20 mg/kg	119 ± 20 [b]	13	178 ± 12	13
30 mg/kg	72 ± 24 [b]	8	181 ± 21	7
L-DOPA + carbidopa				
10 mg/kg + 50 mg/kg	182 ± 15	11	201 ± 10	12
30 mg/kg + 50 mg/kg	163 ± 22	6	161 ± 19	9
50 mg/kg + 50 mg/kg	143 ± 15	20	177 ± 11	20
Entacapone				
30 mg/kg	158 ± 16	8	181 ± 21	8
50 mg/kg	154 ± 13	13	181 ± 12	15
Tolcapone				
10 mg/kg	188 ± 8	8	202 ± 7	8
30 mg/kg	153 ± 9	15	183 ± 13	13
50 mg/kg	169 ± 6	14	181 ± 12	15

The drugs were given i.p. 24, 5 and 1 h before the 5-min swimming test. Mean ± S.E.M., *n* = number of animals. Statistics: [b] $P < 0.05$ versus corresponding vehicle control.

daily) and L-DOPA/carbidopa (50/50 mg/kg, twice daily) were moving actively most of the time (without light signal or electric foot shock) from one compartment to other, whereas the other half of the rats escaped the shocks like the desipramine-treated rats.

3.3. Locomotor activity

Neither entacapone (505 ± 22, mean ± S.E.M., n = 10) nor tolcapone (470 ± 27, n = 10) alone (10 mg/kg), given in the same regimen as in forced swimming test, affected the locomotor activity in rats (control, 462 ± 11, n = 20). The combination of L-DOPA and carbidopa (50/50 mg/kg) markedly reduced the activity of rats (241 ± 22, $P < 0.05$, n = 10). Additional treatment with entacapone (3 and 10 mg/kg; 247 ± 41 and 153 ± 32, respectively, n = 10) or tolcapone (3 and 10 mg/kg; 307 ± 37 and 248 ± 35, n = 10) did not modify the effect of L-DOPA and carbidopa combination. Changes in rearing were similar (not shown).

4. Discussion

In the present study, the pretreatment of rats with catechol-*O*-methyltransferase inhibitors alone did not cause any change in the forced swimming test (tolcapone and entacapone) and learned helplessness paradigm (tolcapone). However, the co-administration of L-DOPA with either catechol-*O*-methyltransferase inhibitor caused an apparent antidepressant-like effect. Even entacapone, blocking catechol-*O*-methyltransferase mainly in the peripheral tissues, with L-DOPA increased the performance of rats in the forced swimming test. It is notable that L-DOPA per se was ineffective up to the dose of 50 mg/kg. The combination of L-DOPA and catechol-*O*-methyltransferase inhibitors did not increase the motor activity of rats but rather reduced it. Thus, the improved performance of animals is not due to the increased motor activity. Interestingly, Moreau and coworkers (1994) have recently established that tolcapone displays the antidepressant-like action in rats. Namely, tolcapone (10 or 30 mg/kg twice daily), without L-DOPA, reversed the anhedonic state induced by chronic mild stress. This result confirms the view that catechol-*O*-methyltransferase inhibition may be of importance in alleviation of depression. In our depression models, the role of L-DOPA seems critical. Catechol-*O*-methyltransferase inhibitors appear to potentiate the effect of L-DOPA. However, the direct central action of catechol-*O*-methyltransferase inhibitors is not wholly excluded since the differences between the brain penetration of entacapone and tolcapone is only relative. High doses of entacapone (30–100 mg/kg) decrease the striatal homovanillic efflux and increase dihydroxyphenylacetic acid efflux in the in vivo microdialysis studies (Kaakkola and Wurtman, 1992; Kaakkola et al., 1994).

L-DOPA treatment increases preferably brain dopamine levels, but also noradrenaline levels are elevated in several brain regions, notably in the cerebral cortex and hippocampus (Chalmers et al., 1971). After L-DOPA treatment, inhibitors of catechol-*O*-methyltransferase may further enhance brain noradrenaline levels. Therefore noradrenergic contribution to the antidepressive effect of L-DOPA and catechol-*O*-methyltransferase inhibitors cannot be ruled out.

However, the dopamine hypothesis of depression, proposed by Randrup and coworkers (1975), is even more attractive. Several studies have revealed an apparent deficiency of dopaminergic neurotransmission in the case of depressive illness as well as in the animal models of depression (Willner, 1985). Moreover, there is a growing body of evidence that dopamine precursors, dopamine receptor agonists and dopamine reuptake inhibitors exert beneficial activity in the animal models of depression. Intermittent administration of dopamine receptor agonists induced an antidepressant-like effect. Also the electroconvulsive therapy, the efficient therapy of deep depression, facilitates dopaminergic function in the brain (Willner, 1985).

From the neuroanatomical point of view, the mesolimbic dopaminergic system could be a target for the action of antidepressive drugs. Indeed, the long-term treatment of rats with desipramine and citalopram or exposure of rats to electroconvulsive therapy seems to increase the dopaminergic transmission in the nucleus accumbens. Namely, after these medications the microinjection of dopaminergic agonists into the nucleus accumbens markedly increases the exploratory activity and improves the performance of rats in the forced swimming test (Plaznik and Kostowski, 1987). Repeated treatment with tricyclic antidepressants reverses the suppression of dopaminergic activity induced by chronic exposure of rats to a variety of mild, unpredictable stressors (Sampson et al., 1991). The facilitation of dopaminergic activity appears to be related to both the increase of sensitivity of postsynaptic dopamine receptors and to the decreased sensitivity of the inhibitory presynaptic dopamine receptors (Maj, 1990).

The present findings may be of importance while using catechol-*O*-methyltransferase inhibitors as adjunct drugs in the L-DOPA treatment of Parkinson's disease. Clinical studies with patients having Parkinson's disease demonstrated that approximately one-third of them has depression (Cummings, 1992). The quality of life of those patients may be greatly improved as revealed by preliminary clinical experiences (for review, see Kaakkola et al., 1994; Männistö, 1994).

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