L-threo-3,4-dihydroxyphenylserine, a noradrenaline precursor, inhibits dopamine release and metabolism in the rat striatum in vivo

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Abstract. The effect of L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS) on dopamine (DA) release and metabolism in the striatum was studied in freely moving rats by intracerebral microdialysis techniques. The DA level as well as the levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid were significantly decreased 140 min after the administration of L-threo-DOPS (50 mg/kg intraperitoneally). The results suggest that L-threo-DOPS inhibits the release and metabolism of DA in the striatum.
Key words. In vivo microdialysis; striatum; noradrenaline; L-threo-3,4-dihydroxyphenylserine; dopamine release; 3,4-dihydroxyphenylacetic acid; homovanillic acid; high performance liquid chromatography.

It has been reported that a marked decrease of both dopamine-β-hydroxylase (DBH) and tyrosine hydroxylase (TH) activities occur in the brain tissue of Parkinsonian patients[1]. This suggests that lesions not only in dopaminergic but also in noradrenergic neuronal systems in the CNS might be involved in this disease. Narabayashi et al.[2] reported that L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS), that was directly converted to noradrenaline (NA) by aromatic L-amino acid decarboxylase (AADC) without a DBH intermediate, could ameliorate the freezing symptom. This is often a disabling symptom in Parkinsonian patients with long-standing disease, even when the rigidity and tremor have been well controlled over a long period by L-DOPA. However, little is known about the influence of this drug on dopaminergic neuronal activity in the striatum. In the present study, we investigated the influence of L-threo-DOPS on dopamine (DA) release and metabolism in the rat striatum by measuring the levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in this region, using the brain microdialysis method[3,4].

Materials and methods

Male Wistar rats weighing 250–300 g were used. Animals were maintained on a 12 h light/dark cycle (lights on at 07.00 h) with free access to food and water. Rats were anesthetized with pentobarbital (45 mg/kg i.p.) and the dialysis probe was implanted into the striatum (coordinates were A: 0.5 mm, L: 3.0 mm, V: 7.0 mm, from the bregma and the dura surface according to the atlas of Paxinos and Watson[5]. After the experiment, the position of the dialysis probe was verified by anatomical examination.

A dialysis experiment was carried out one day after the implantation of the probe. The dialysis probe was continuously perfused at a constant flow rate of 2.5 µl/min with an artificial CSF replacement (NaCl 140 mM, KCl 3.35 mM, $MgCl_2$ 1.15 mM, $CaCl_2$ 1.26 mM, Na_2HPO_4 1.20 mM, NaH_2PO_4 0.3 mM and pH 7.4).

About 3 h after the beginning of the perfusion, stable basal levels of DA, DOPAC and HVA in the dialysates were obtained. Then L-threo-DOPS suspended in 0.3% carboxymethylcellulose sodium (CMC) solution was administered i.p. at 50 mg/kg and the levels of DA, DOPAC and HVA were measured until 5 h after the injection.

DA, DOPAC and HVA were measured by injecting the dialysates injected into the high performance liquid chromatography coupled to electrochemical detection (HPLC-ECD) system (EICOM Co., Kyoto, Japan). The mobile phase was 0.1 M sodium acetate buffer (pH 4.5) containing 2.5–3.3 mM octanesulfonic acid, 0.02 mM EDTA and 10.0% (v/v) methanol. The column (4.6 × 150 mm, 7 µ ODS-C18 resin, EICOM Co.) was used at 25 °C. The graphite working electrode was set at + 600 mV vs a Ag/AgCl reference electrode (EICOM ECD-100 electrochemical detector) and the flow rate (EICOM EP-10 pump) was 0.90 ml/min. Statistical analysis of the data was evaluated by one-way analysis of variance followed by Student's t-test.

The averaged basal levels of DA, DOPAC and HVA were determined from the mean of 3 samples obtained prior to injection. The identity of the DA peak was confirmed by addition of TTX (5×10^{-7} M) to the perfusion fluid, which caused a disappearance of the DA peak. Basal levels of DA, DOPAC and HVA in the striatum were 49 ± 9 pg/20 min (n = 4), 6674 ± 711 pg/20 min (n = 4) and 3169 ± 856 pg/20 min (n = 4), respectively. No remarkable changes in DA or its metabolites were seen in the vehicle-injected (control) group, but in the group given L-threo-DOPS (50 mg/kg i.p.) the level of DA as well as the levels of DOPAC and HVA were significantly decreased 140 min after the injection (figs 1 and 2). The decrease in the striatal levels of DA, DOPAC and HVA in response to the administration of L-threo-DOPS was time-dependent until 5 h after the injection.

This is the first report demonstrating directly that L-threo-DOPS inhibits both DA release and metabolism in the rat striatum in vivo. A decrease in DA level after the administration of L-threo-DOPS was observed in a clinical report on a DBH deficiency[6]: the plasma DA level was decreased and NA level was increased after the treatment with L-threo-DOPS. In this case, it was speculated

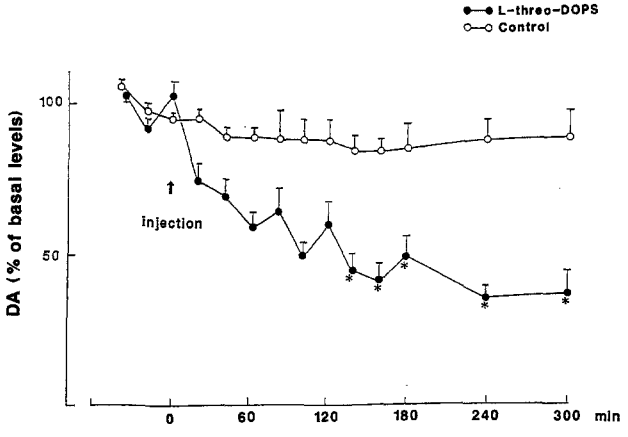

Figure 1. Effect of L-threo-DOPS on DA release in striatum. Values are indicated as means ± SEM of 4 animals. *p < 0.05 vs basal levels.

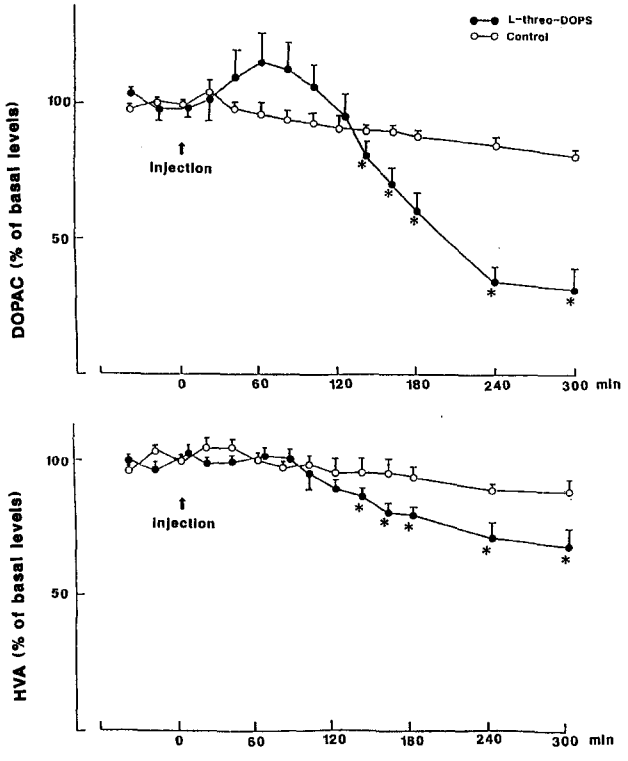

Figure 2. Effect of L-threo-DOPS on DA metabolism in striatum. Values are indicated as means ± SEM of 4 animals. *p < 0.05 vs basal levels.

that the increased NA level might inhibit the TH activity, thus reducing the production of DA. There is in vitro work to support the speculation that TH activity in corpus striatal extracts was inhibited by NA[7]. In addition some in vitro work[8] shows that the administration of one precursor lowered the level of other neurotransmitters in the brain. In this case, administration of L-dopa decreased brain serotonin level. This finding suggested that the decreased serotonin level was due to displacement of serotonin by the newly formed DA. Based on the above findings, we suggest that there are two mechanisms which may cause a decrease in DA levels after the administration of L-threo-DOPS. L-threo-DOPS may be taken up into striatal dopaminergic neurons, instead of being into noradrenergic neurons which are very sparsely innervated in this area[9] and converted to NA via AADC in dopaminergic neurons. It could also be that synthesized NA inhibits TH activity in dopaminergic neurons although the extracellular NA level was too small to be detected in the present study. These mechanisms might result in the reduction of DA release in this brain region. The present data suggest that, by inhibiting DA neuronal activities in the striatum, L-threo-DOPS might be useful in the treatment of Parkinsonian patients.

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