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Studies on the Central Action of L-threo-3,4-dihydroxyphenyl-serine (L-threo-DOPS) in FLA-63-Treated Mice

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KATO, T., M. KATSUYAMA, N. KARAI, M. NAKAMURA AND J. KATSUBE. *Studies on the central action of L-threo-3,4-dihydroxyphenyl-serine (L-threo-DOPS) in FLA-63-treated mice.* PHARMACOL BIOCHEM BEHAV **26**(2) 407–411, 1987.—In order to clarify the central action of L-threo-DOPS, the effect of benserazide on behavioral and biochemical changes by L-threo-DOPS in FLA-63-treated mice was studied. L-threo-DOPS in combination with nialamide markedly increased both the locomotor activity and the concentrations of the brain, heart and kidney norepinephrine (NE) in the FLA-63-treated mice. Benserazide at low doses did not alter either the rise of the brain NE level or the increase in locomotor activity, whereas it significantly inhibited the rise of the heart and kidney NE levels. Benserazide at a high dose significantly inhibited all of them. These results suggested that the increase in locomotor activity might be mediated via activation of the central noradrenergic neurons system by L-threo-DOPS.

L-threo-DOPS Norepinephrine FLA-63 Locomotor activity Benserazide

L-DOPA therapy for Parkinson's disease (PD) is well established as the compensation therapy for dopamine (DA) deficiency in the brain tissue. L-threo-3,4-dihydroxyphenyl-serine (L-threo-DOPS), a norepinephrine (NE) precursor, has recently been shown to have beneficial effects on the freezing phenomenon or akinesia in PD [15,16]. Lowering of both brain NE levels and DA-β-hydroxylase (DBH) activity has also been demonstrated in advanced parkinsonian patients [4,14]. Thus L-threo-DOPS is postulated to have beneficial effects on PD by compensating the NE deficiency in the brain.

So, we have attempted to define whether L-threo-DOPS may replenish the depleted brain NE level using FLA-63 (a DBH inhibitor) -treated mice; an NE-depleted animals model, although a couple of studies with the FLA-63-treated animals models have already been reported by Svensson [21], Ahlenius and Engel [1], Edwards and Rizk [7] and Semba and Takahashi [18].

In the present study, we have investigated the central action of L-threo-DOPS in combination with benserazide in FLA-63-treated mice.

METHOD

Animals

Male dd strain mice, weighing 23–28 g, were used for all studies. All experiments were performed at 24±1°C and a relative humidity 55±5%.

Determination of Locomotor Activity

Benserazide (an extracerebral decarboxylase inhibitor), nialamide (a monoamineoxidase inhibitor) and L-threo-DOPS were administered intraperitoneally (IP) 30 min, 60 min and 90 min, respectively, after the IP injectin of FLA-63 (40 mg/kg). The animals were put 5 at a time in the Automex (Columbus Instruments, Columbus, OH) cages immediately after L-threo-DOPS. Thirty min after L-threo-DOPS, the locomotor activity was measured for 60 min.

Determination of NE

Biochemical analyses were performed after the same treatment schedule and these animals were killed by decapitation 150 min after FLA-63. The brain, heart or kidney NE was measured according to the method of a previous paper [11].

Drugs

L-threo-DOPS was synthesized in the laboratory of Sumitomo Pharmaceuticals Co. Ltd. L-threo-DOPS and FLA-63 (Sigma, St. Louis, MO) were suspended in 0.5%

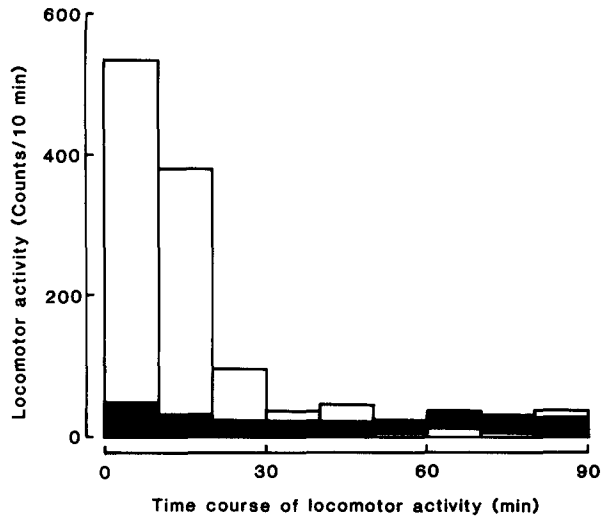

FIG. 1. Time course of locomotor activity with or without FLA-63 in mice. The locomotor activity was measured for 90 min, 90 min after the intraperitoneal injection of FLA-63 (40 mg/kg). ☐ Normal mice; ▩ FLA-63-treated mice; N=5, N=Number of experiments.

methylcellulose solution. Nialamide (Sigma, St. Louis, MO) was dissolved in a minimum quantity of 0.1 N hydrochloride solution made up to volume with distilled water. Benserazide (Hoffman-La Roche, Basel, Switzerland) was dissolved in distilled water.

Statistics

Results were expressed as mean±s.e.m. and the statistical significance was determined by Student's *t*-test.

RESULTS

The locomotor activity in mice for 30 min, 90 min after FLA-63 was markedly decreased, compared with that of control (FLA-63-treated mice, 148±26 counts; normal mice, 1246±313 counts). After 30 min, however, there was no difference in the locomotor activity between the two because of adaptation (Figs. 1 and 2), whereas the brain NE level at 150 min after FLA-63 was significantly decreased, compared with the control level (Table 1).

L-threo-DOPS (400 mg/kg) or nialamide (30 mg/kg) alone did not alter the locomotor activity in normal mice (Fig. 2). Moreover, these drugs at the same doses did not alter either the locomotor activities or the brain NE levels in the FLA-63-treated mice. On the other hand, L-threo-DOPS (400 mg/kg) plus nialamide (30 mg/kg) markedly increased not only the locomotor activity but also the depleted brain, heart and kidney NE levels (Figs. 2–4 and Table 1).

Benserazide at low doses (0.1 and 0.3 mg/kg) did not alter either the increase in the locomotor activity or brain NE level by L-threo-DOPS in combination with nialamide in the FLA-63-treated mice, whereas the increase in the heart and kidney NE levels were significantly inhibited (Figs. 3 and 4). Benserazide at a high dose (1 mg/kg) on the other hand, diminished not only the rise of NE in the peripheral organs (heart and kidney), but also that in the brain (Fig. 4). Furthermore, the increase in the locomotor activity was also diminished (Fig. 3).

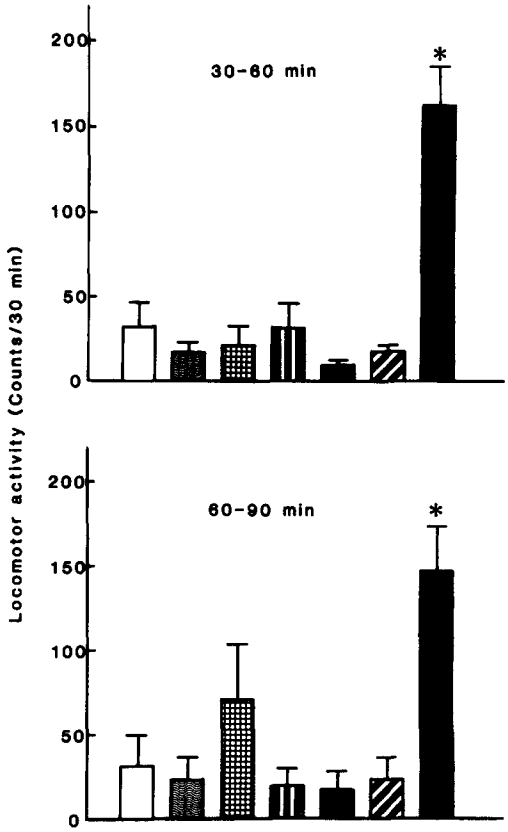

FIG. 2. The increase in locomotor activity in FLA-63-treated mice induced by L-threo-DOPS plus nialamide. Nialamide (30 mg/kg) and L-threo-DOPS (400 mg/kg) were injected intraperitoneally 60 min and 90 min, respectively, after the intraperitoneal injection of FLA-63 (40 mg/kg). Thirty min after L-threo-DOPS or its vehicle, the locomotor activity was measured for 60 min. ☐ Control; N=6, ▩ L-DOPS; N=6, ▩ nialamide; N=6, ▮▮ FLA-63; N=6, ■ FLA-63 + L-threo-DOPS; N=6, ▨ FLA-63 + nialamide; N=6, ■ FLA-63 + L-threo-DOPS + nialamide; N=6. N=number of experiments. *$p<0.05$; compared with FLA-63 alone (Student's *t*-test).

DISCUSSION

FLA-63 markedly inhibits DBH and lowers the brain NE level, and causes the decrease in locomotor activity or hypothermia in experimental animals [20,21]. We also confirmed that FLA-63 decreased the brain NE level as well as locomotor activity in mice. In this paper, the locomotor activity in mice was measured for the period (60 min), from 120 to 180 min after the injection of FLA-63, in order to assess only the spontaneous locomotor activity excluding exploratory behavior. Nialamide or L-threo-DOPS alone did not alter either the brain NE level or locomotor activity in the FLA-63-treated mice. L-threo-DOPS plus nialamide markedly increased not only the depleted brain NE level but also locomotor activity. These results are in agreement with that of Svensson [21].

Friedman and Gershon [9] have reported that L-

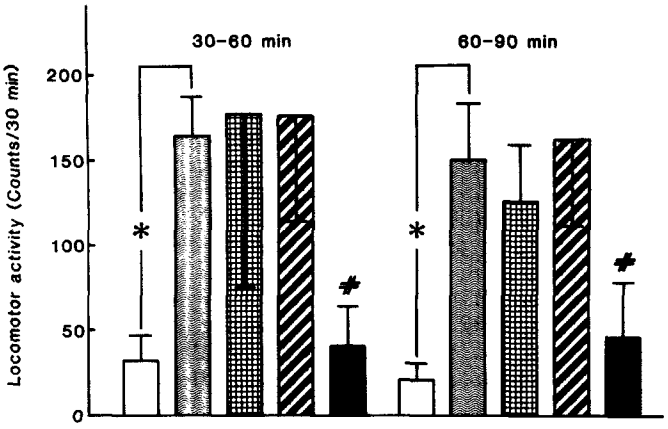

FIG. 3. Effect of benserazide on the increase in locomotor activity by L-threo-DOPS and nialamide in FLA-63-treated mice. Benserazide, nialamide (30 mg/kg) and L-threo-DOPS (400 mg/kg) were injected intraperitoneally 30 min, 60 min and 90 min, respectively, after the intraperitoneal injection of FLA-63 (40 mg/kg). Thirty min after L-threo-DOPS, the locomotor activity was measured for 60 min. ☐ FLA-63; N=6, ≋ FLA-63 + L-threo-DOPS + nialamide; N=6, ▦ FLA-63 + L-threo-DOPS + nialamide + benserazide (0.1 mg/kg); N=6, ▨ FLA-63 + L-threo-DOPS + nialamide + benserazide (0.3 mg/kg); N=6. ■ FLA-63 + L-threo-DOPS + nialamide + benserazide (1.0 mg/kg); N=6. N=Number of experiments. *$p<0.05$; compared with FLA-63 alone, #$p<0.05$; compared with FLA-63 + L-threo-DOPS + nialamide (Student's t-test).

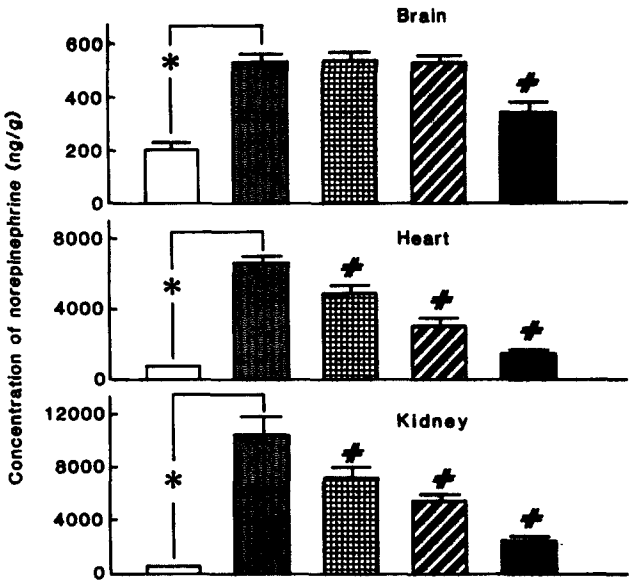

FIG. 4. Effect of benserazide on the increase in brain, heart and kidney concentrations of norepinephrine induced by L-threo-DOPS and nialamide. Benserazide, nialamide and L-threo-DOPS (400 mg/kg) were injected intraperitoneally 30, 60 and 90 min, respectively, after the intraperitoneal injection of FLA-63 (40 mg/kg). The animals were killed 60 min after L-threo-DOPS. ☐ FLA-63; N=5, ≋ FLA-63 + L-threo-DOPS + nialamide; N=5, ▦ FLA-63 + L-threo-DOPS + nialamide + benserazide (0.1 mg/kg); N=5, ▨ FLA-63 + L-threo-DOPS + nialamide + benserazide (0.3 mg/kg); N=5, ■ FLA-63 + L-threo-DOPS + benserazide (1.0 mg/kg); N=5. N=Number of experiments. *$p<0.05$; compared with FLA-63 alone, #$p<0.05$; compared with FLA-63 + L-threo-DOPS (Student's t-test).

DOPA-induced hyperactivity in rats was potentiated by a tricyclic anti-depressant, imipramine, and concluded that the motor activity was primarily mediated by brain NE, since imipramine inhibited the uptake of NE, but not of DA, into aminergic neurons of the brain [10]. It has also been shown that the locomotor activity was increased by NE injected bilaterally into the rat nucleus accumbens [5]. These data indicate that the locomotor activity is increased by the activation of central noradrenergic neurons system. Thus the increase in locomotor activity in the FLA-63-treated mice by L-threo-DOPS plus nialamide may be due to the increase in the brain NE content by L-threo-DOPS. However, there is a possibility that this action of L-threo-DOPS may be due to NE formed from L-threo-DOPS in the periphery, since L-threo-DOPS plus nialamide markedly increased the peripheral NE levels. Such a possibility has also been pointed out in some previous papers [2,18].

So, in order to clarify the central action of L-threo-DOPS, the effect of benserazide on the action of L-threo-DOPS was studied. In our other experiments using normal mice, it was found that the effective dosage of benserazide as a peripheral decarboxylase inhibitor was a small amount (around 1 mg/kg) for inhibition of L-threo-DOPS decarboxylation [12], whereas a large amount of benserazide was found to be necessary for that of L-DOPA decarboxylation, as suggested by Bartholini *et al.* [2]. Benserazide (1 mg/kg) alone and combined with only L-threo-DOPS (400 mg/kg) did not modify the FLA-63-induced effects (data not shown). However, benserazide at low doses (0.1 and 0.3 mg/kg) significantly inhibited the increases in the heart and kidney NE levels, whereas it did not alter either the increase in the brain NE level or locomotor activity by L-threo-DOPS plus nialamide

TABLE 1

THE INCREASE IN THE BRAIN CONCENTRATION OF NOREPINEPHRINE INDUCED BY L-THREO-DOPS AND NIALAMIDE IN FLA-63 TREATED MICE

Drugs	mg/kg (IP)	Brain Concentration of Norepinephrine (ng/g)
Control	—	513 ± 11
FLA-63	40	206 ± 29*
FLA-63	40	
+ Nialamide	30	213 ± 14
FLA-63	40	
+ L-threo-DOPS	400	200 ± 5
FLA-63	40	
+ Nialamide	30	
+ L-threo-DOPS	400	528 ± 29†

Nialamide and L-threo-DOPS were injected intraperitoneally 60 min and 90 min, respectively, after the intraperitoneal injection of FLA-63. The animals were killed 60 min after L-threo-DOPS. *$p<0.05$; compared with control, †$p<0.05$; compared with FLA-63 alone (Student's t-test). N=5; Number of experiments. Values are expressed as mean ± s.e.m.

in the FLA-63-treated mice. Accordingly, the increase in locomotor activity by L-threo-DOPS in combination with nialamide appears to be related to NE formed from L-threo-DOPS in the brain.

Benserazide at a high dose (1 mg/kg), on the other hand, diminished not only the rise of NE in peripheral organs (heart and kidney), but also that in the brain. Moreover, the locomotor activity was also diminished. Svensson [21] reported that the peripheral injection of NE did not antagonize the FLA-63-induced effects. Furthermore, since benserazide at a dose of 2.5 mg/kg has been reported to inhibit the striatal decarboxylase activity by about 20% [13], some portion of benserazide at such a dose (1 mg/kg and more) would possibly penetrate through the blood-brain barrier into the brain and inhibit decarboxylation of L-threo-DOPS there. These results suggest that the increase in locomotor activity by L-threo-DOPS plus nialamide may be due to NE formed from L-threo-DOPS in central noradrenergic neurons and support the view that the increase in the brain NE content may be attributed to the rise of locomotor activity [9].

On the other hand, it has been reported that central dopaminergic neurons are involved in the control of motor activity, since L-DOPA or amphetamine produced the increase in locomotor activity in mice and rats [3, 6, 17] and the hyperactivity was induced by dopamine or amphetamine injected bilaterally into the rat nucleus accumbens [5]. In addition, Edwards and Sedlock [8] reported that L-threo-DOPS produced the increase in the brain level of DA metabolite (HVA) in normal rats. Thus, such a possibility has to be taken into consideration as that not only noradrenergic neurons system, but also dopaminergic neurons system, may be involved in the L-threo-DOPS-induced increase in locomotor activity in the FLA-63-treated mice. However, Svensson [21] reported that the brain DA content after L-threo-DOPS plus nialamide in the FLA-63-treated mice was not affected. Moreover, it was found in our experiments that L-threo-DOPS did not alter either the brain DA content [12] or DA metabolites (DOPAC, HVA) in normal mice (normal mice: DOPAC 98.4±3.5 ng/g; HVA 144±6 ng/g, L-threo-DOPS 400 mg/kg; IP: DOPAC 105±4 ng/g; HVA 160±12 ng/g). Therefore, dopaminergic neurons system would not be primarily attributed to the antagonism induced by L-threo-DOPS plus nialamide in the FLA-63-treated mice.

Accordingly, L-threo-DOPS could at least in part pass through the blood-brain barrier and would be converted to NE at the synaptic sites of central noradrenergic neurons. Thus L-threo-DOPS appears to be an effective NE precursor in the brain.

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