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The Effect of L-Erythro-Dihydroxyphenylserine Injected Into the Lateral Ventricle and the Hypothalamus on the Locomotor Activity

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NOTO, T., H. HASHIMOTO, J. NAKAO, H. KAMIMURA, T. MORI AND T. NAKAJIMA. *The effect of L-erythro-dihydroxyphenylserine injected into the lateral ventricle and the hypothalamus on the locomotor activity.* PHARMACOL BIOCHEM BEHAV **25**(2) 411–414, 1986.—The effect of dihydroxyphenylserine (DOPS) on locomotor activity was studied using the Animex activity meter. One μg of L-erythro-DOPS, a precursor of d-noradrenaline, was injected into the lateral ventricle once a day for one week or into the anterior hypothalamic area (AHA) once. After the intraventricular injection, the total locomotor activity (from 8:00 p.m. to 8:00 a.m.) of the rats injected with DOPS was significantly less than that of the rats injected with an artificial cerebrospinal fluid (5-ion). Analysis of the locomotor activity in consecutive 2-hr periods showed that the activity of the DOPS group during the time intervals of 10 p.m.–12 a.m., 12 a.m.–2 a.m. and 2 a.m.–4 a.m. was significantly less than that of the control group. After injection of DOPS or 5-ion into AHA, the total activity of the DOPS group was significantly less than that of the control. Analysis of the activity of the DOPS group for each 2-hr period between 10 p.m.–4 a.m. was also significantly less than that of the control. On the basis of these findings, the effect of DOPS in the brain noradrenergic system are discussed.

DOPS Locomotor activity Noradrenaline Lateral ventricle Hypothalamus Brain

MANIA has been considered to be due to a hyperactivity of the noradrenergic system in the central nervous system (CNS) [14–16]. Therefore, agents suppressing activity of the noradrenergic system in the CNS might be effective in controlling manic excitement. However, we know of few drugs that specifically suppress the noradrenergic system in the CNS. If d-noradrenaline, an unnatural amine, could be increased in the CNS, it might displace l-noradrenaline in the CNS [13] and specifically suppress the noradrenergic system.

For the purpose of controlling manic excitement by directly suppressing l-noradrenergic receptors in the CNS, L-erythro-DOPS was synthesized (Sumitomo Kagaku Kogyo Co.). DOPS is thought to be a direct precursor of noradrenaline, but not naturally occurring. DOPS has 4 isomers, L-erythro-DOPS, D-erythro-DOPS, L-threo-DOPS, and D-threo-DOPS and only the L-types are known to be metabolized to noradrenaline by L-aromatic amino acid decarboxylase in the mammal. L-erythro-DOPS has been reported to be metabolized to d-noradrenaline and L-threo-DOPS has been confirmed to be metabolized to l-noradrenaline [2,12] which is known to be a neurotransmitter in the CNS. However, L-threo-DOPS has been reported not to be a good substrate for L-aromatic amino acid decarboxylase [6]. On the other hand, L-erythro-DOPS has been shown to be such a good substrate for the enzyme that L-erythro-DOPS was 20 times more rapidly decarboxylated than L-threo-DOPS [6]. Recently we demonstrated the suppressive effect of DL-erythro-DOPS injected intraperitoneally on the mouse locomotor activity which had previously been enhanced by an injection of MAO inhibitor or amphetamine [10].

In this report, we demonstrate the suppressive effect of L-erythro-DOPS, when injected into the lateral ventricle and the hypothalamus of rat brain, on spontaneous locomotor activity.

METHOD

Chemicals

L-erythro-DOPS was supplied from Sumitomo Kagaku Kogyo Co. in Japan.

Animals and Surgery

Male Wistar rats weighing 250–300 g were housed in individual cages at a room temperature of 24–28°C. Each

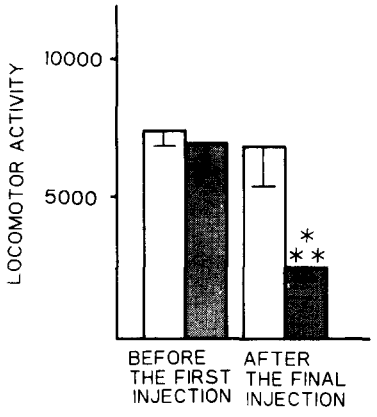

FIG. 1. The locomotor activity of rats before and after the one week injection of DOPS or 5-ion into lateral ventricle (M±SEM). Open columns: locomotor activity of rats injected with 5-ion (n=3). Closed columns: locomotor activity of rats injected with DOPS (n=3). *$p<0.05$. The locomotor activity after the one week injection of DOPS was significantly less than that of 5-ion. **$p<0.05$. The locomotor activity after the one week injection of DOPS was significantly less than that before the injection of DOPS.

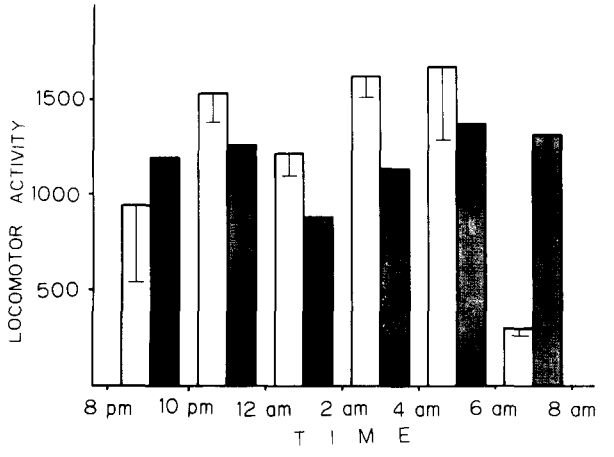

FIG. 2. The locomotor activity of rats on each 2 hr from 8:00 p.m. to 8:00 a.m. before the one week injection of DOPS or 5-ion (M±SEM). Open columns: locomotor activity of rats injected with 5-ion (n=3). Closed columns: locomotor activity of rats injected with DOPS (n=3).

animal was maintained on a 12 hr light cycle (light-on, 8:00 p.m. to 8:00 a.m.) with food and water available ad lib.

Each rat was anesthetized with 40 mg/kg sodium pentobarbital given intraperitoneally. Twenty-three gauge stainless steel guide tubes were then implanted bilaterally with the tips positioned above the intended sites at stereotaxic coordinates, AP: 5.6, Lat: 1.6, HV:4.6 and AP:6.2, Lat:0.8, HV:4.6 mm [10]. A stylet was kept within the guide tube and a protective cap was screwed onto the pedestal to prevent damage. A minimum of one week elapsed before the experiments began.

Intraventricular Infusion of DOPS

One μg of L-erythro-DOPS dissolved in a volume of 1 μl of an artificial cerebrospinal fluid [11] or 5-ion was injected into the lateral ventricle of an unanesthetized rat through the previously implanted guide cannula using 0.3 mm (outer diameter) stainless steel injection cannula which was connected to a polyethylene tube (PE 10) filled with DOPS solution or 5-ion. The tip of the injector needle was inserted into the lateral ventricle and then the volumetrically-calibrated polyethylene tube was lifted vertically. L-erythro-DOPS or 5-ion was administered at approximately 5:00 p.m. Each rat was injected with L-erythro-DOPS or 5-ion once a day for 1 week.

Hypothalamic Infusion of DOPS

One μg of L-erythro-DOPS dissolved in a volume of 0.5 μl of 5-ion or the same volume of 5-ion was injected into the anterior hypothalamic area (AHA) of an unrestrained rat through the implanted guide tube using 0.3 mm (outer diameter) stainless steel injection cannula that was connected to 10 μl microsyringe with a polyethylene tube (PE 10) filled with DOPS solution or 5-ion. DOPS or 5-ion was injected at approximately 5:00 p.m. only one time at the injection rate of 0.5 μl/5 min.

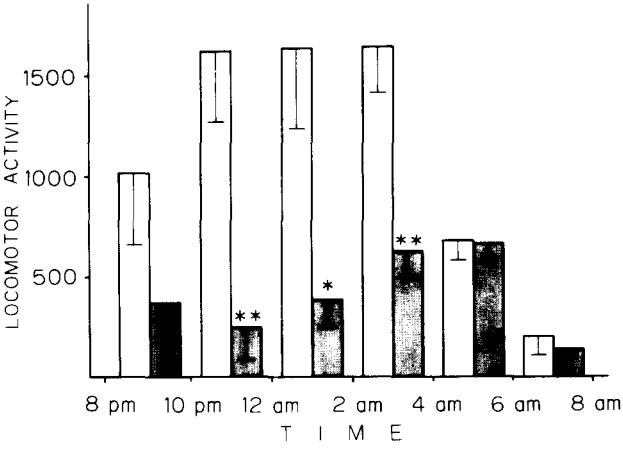

FIG. 3. The locomtor activity of rats on each 2 hr from 8:00 p.m. to 8:00 a.m. after the one week injection of DOPS or 5-ion (M±SEM). Open columns: the locomotor activity of rats injected with 5-ion (n=3). Closed columns: the locomotor activity of rats injected with DOPS (n=3). *$p<0.05$. The locomotor activity of rats injected with DOPS was significantly less than those injected with 5-ion. **$p<0.025$. The locomotor activity of rats injected with DOPS was significantly less than those injected with 5-ion.

Determination of the Locomotor Activity of a Rat Injected With 5-Ion or DOPS

In the case of the intraventricular injection, locomotor activity was measured before the first injection and after the final injection of the week-long series. In the case of the AHA injection, it was counted before and after the single injection. For measuring the locomotor activity, the rat was moved to the transparent plastic cage on the Animex activity meter (type SE, LKB-Farad) which was connected to an Animex printing counter, type 1-X-0. The sensitivity of the Animex activity meter was set at 35 μA. Locomotor activity was counted at intervals of 10 min from 6:00 p.m. to 9:00 a.m.

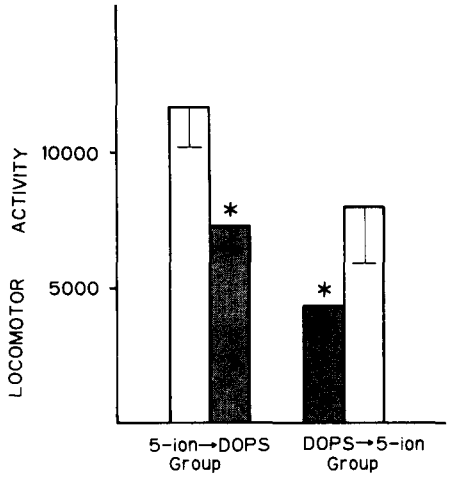

FIG. 4. The total locomotor activity of rats injected with 5-ion and then DOPS (5-ion-DOPS group) and DOPS and then 5-ion (DOPS-5-ion group) into AHA from 8:00 p.m. to 8:00 a.m. (M±SEM). Open columns: the locomotor activity of rats injected with 5-ion (n=4). Closed columns: the locomotor activity of rats injected with DOPS (n=4). $*p<0.05$. In both groups the total locomotor activities of rats injected with DOPS were significantly less than those injected with 5-ion.

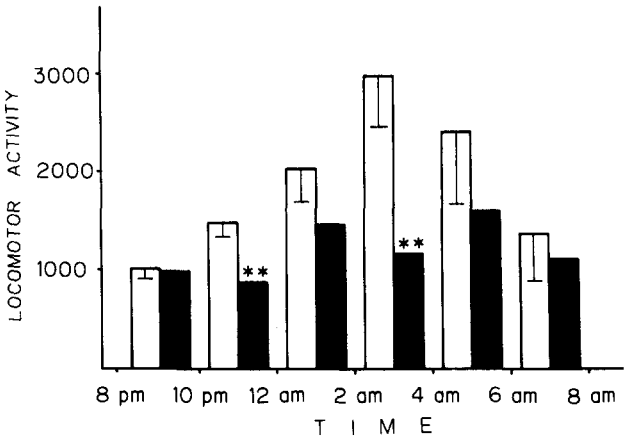

FIG. 5. The locomotor activity of rats injected with 5-ion and then DOPS on each 2 hr from 8:00 p.m. to 8:00 a.m. (M±SEM). Open columns: the locomotor activity of rats injected with 5-ion (n=4). Closed columns: the locomotor activity of rats injected with DOPS (n=4). $**p<0.025$. The locomotor activity of rats injected with DOPS was significantly less than those injected with 5-ion.

Histological Analysis of the Infusion Site and Statistical Analysis

At the completion of the experiments, the brain was perfused with saline and 10% formalin and then was removed. Serial sections of the perfused brain were made and stained. The anatomical area of the perfused site was traced under a light microscope.

The mean value and standard error of locomotor activity was calculated and Student's *t*-test (two-tail) was performed to compare the activity of control and DOPS groups.

RESULTS

The Effect of L-Erythro-DOPS Injected Into the Lateral Ventricle on Locomotor Activity

The total locomtor activities of DOPS and control groups from 8:00 p.m. to 8:00 a.m. were analyzed before and after the one week injections. Before the injection there were no significant differences in total activity between the DOPS and control groups; for the control group, the activity before the first injection of 5-ion was also not significantly different from that after the final injection (Fig. 1). In contrast, the total activity of the DOPS groups after the final injection was significantly different from that of the control and in the DOPS group, the activity after the final injection was significantly less than that before the first injection (Fig. 1).

The locomotor activity before and after the injection was measured in every 2 hr period from 8:00 p.m. to 8:00 a.m. Before the injection of DOPS or 5-ion for each 2-hour period, no significant differences were found between the activities of DOPS and control groups (Fig. 2). However, after the injection the activity of the DOPS group was significantly less than that of the control group at 10:00 p.m.–12:00 a.m., 12:00 a.m.–2:00 a.m., and 2:00 a.m.–4:00 a.m. (Fig. 3).

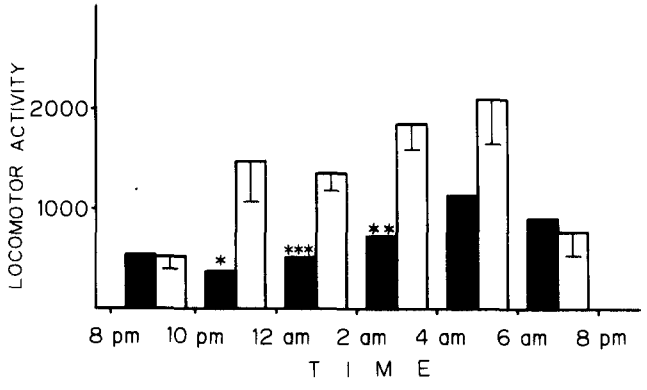

FIG. 6. The locomotor activity of rats injected with DOPS and then 5-ion on each 2 hr from 8:00 p.m. to 8:00 a.m. (M±SEM). Open columns: the locomotor activity of rats injected with 5-ion (n=4). Closed columns: the locomotor activity of rats injected with DOPS (n=4). $*p<0.05$, $**p<0.025$, $***p<0.005$. The locomotor activity of rats injected with DOPS was significantly less than those injected with 5-ion.

The Effect of L-Erythro-DOPS Injected Into the Anterior Hypothalamic Area (AHA) on Locomotor Activity

In the study of AHA, a different experimental procedure was used from that for the lateral ventricle. In this study, 8 rats were divided into 2 groups in which the first group was injected with 5-ion in the first session and then with DOPS in the second session and the second group was injected in a reverse manner with DOPS in the first session followed by the injection with 5-ion in the second session. In both groups, the total locomtor activity when injected with DOPS was significantly less than when injected with 5-ion (Fig. 4).

The activities of the two groups were analyzed in each two-hour period. In the first group, the activity after injec-

tion of DOPS was significantly less at 10:00 p.m.–12:00 a.m., and 2 a.m.–4:00 a.m. than that with 5-ion (Fig. 5). In the second group, the locomotor activity of the rats injected with DOPS was also significantly less at 10:00 p.m.–12:00 a.m., 12:00 a.m.–2:00 a.m., and 2:00 a.m.–4:00 a.m. than after injection of 5-ion (Fig. 6).

Histological Analysis of the Infusion Sites

In the case of AHA injections, the anatomical regions of the injected sites were identified by light microscopy and traced onto histological reconstructions drawn in the coronal plane. The injection sites in the 8 rats were within the AHA. However, in the case of intraventricular injection, the tip of the injection cannula in the one of the 4 rats reached the hippocampus, which was slightly damaged. Therefore, this rat was excluded from the analysis of the locomotor activity.

DISCUSSION

Locomotor activity has been considered to be regulated by the dopaminergic neurotransmission system in the CNS on the basis of the findings that the locomotor stimulation by amphetamine, cocaine, or some other drugs was decreased by treatment with dopamine synthesis inhibitors, dopamine receptor antagonists, or 6-hydroxydopamine [8]. This locomotor stimulation was also considered to be antagonized by the serotonergic and the GABAergic neurotransmitter systems [8]. However, the noradrenergic neuro-transmission system is thought to play no role in locomotor activity [3,7]. On the other hand, other studies suggest a role of the noradrenergic system in locomotor activity which is stimulated following the intraventricular injection of noradrenaline [4,5]; also, reserpine-induced suppression of locomotor activity was counteracted by noradrenaline receptor activation [1]. Moreover, in our previous study, DL-erythro-DOPS injected intraperitoneally decreased the locomotor stimulation induced by MAO-inhibitors or metamphetamine, but the decrease of spontaneous locomotor activity without treatment with a MAO-inhibitor or metamphetamine was very slight [9]. Therefore, the decrease of locomotor activity may involve the suppression of the noradrenergic transmission system. In the present study, L-erythro-DOPS injected into the lateral ventricle and the anterior hypothalamic area decreased the locomotor activity of the rats. Therefore, the noradrenaline transmission system in the brain, especially in the anterior hypothalamic area, is strongly implicated in playing a role in the regulation of locomotor activity.

On the basis of these findings, L-erythro-DOPS may be found useful in managing manic patients in the near future.

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