Increasing CNS norepinephrine levels by the precursor L-DOPS facilitate beam-walking recovery after sensorimotor cortex ablation in rats

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Abstract

The present investigation was conducted to document a role of L-threo-3,4-dihydroxyphenylserine (L-DOPS), precursor of L-norepinephrine (NE), in the functional recovery from beam-walking performance deficits in rats after unilateral sensorimotor cortex ablation. L-DOPS was administered simultaneously with benserazide (BSZ; a peripheral aromatic amino acid decarboxylase inhibitor), and the regional contents of NE in the cerebral cortex, hippocampus, and cerebellum were assayed. Behavioral recovery was demonstrated by the rats treated with L-DOPS and BSZ, and the rate of recovery was significantly different from that of either BSZ-treated or vehicle-treated control rats. The NE tissue levels in the three discrete regions of the rat brain were significantly elevated in the experimental rats receiving both L-DOPS and BSZ. The present studies indicate that increasing NE levels by the precursor L-DOPS may be responsible for facilitating behavioral recovery from beam-walking performance deficits in rats, and further suggest that L-DOPS may become one of the candidate compounds for further clinical human trials promoting functional recovery after injuries to the cerebral cortex.

Keywords: Functional recovery; Norepinephrine; Sensorimotor cortex; Hemiplegia; L-DOPS; Beam-walking performance; Cortex ablation

1. Introduction

Over the past decade, it has been demonstrated that the damaged central nervous system (CNS) has more potential for functional recovery than previously recognized [12,24]. The mechanism of functional recovery following brain damage varies according to the site and size of the lesions and the age at the time of injury. Little is known about the underlying background for the recovery, and to date, its proposed mechanisms have been explained by the theories of neuronal rearrangement, receptor supersensitivity, enzyme hyperactivity, or diaschisis [2,7,12,13,28,29,35]. Determination of true underlying mechanisms of functional recovery after brain damage may be readily associated with clinical application in humans [8,12]. Among pharmacological agents which modulate the rate of recovery from locomotor deficits by influencing neuronal transmission [12], dextro-amphetamine (d-amphetamine) in particular has drawn considerable attention. Its promising effects upon functional recovery were favorably demonstrated in both experimental animal models and human stroke hemiplegic patients [8,10,20,24,37,38].

Because of abuse potential, even experimental use of d-amphetamine is restricted in Japan. In theamphetamine-facilitated beam-walking paradigm, it is accepted that an increased release of L-norepinephrine (NE) in the CNS rather than dopamine (DA) is considered the transmitter mediating this effect [4,12–15]. An in vivo microdialysis study demonstrated that systemic administration of l-threo-3,4-dihydroxyphenylserine (l-DOPS), a NE precursor which readily crosses the blood-brain barrier, produced a prolonged increase in extracellular levels of NE [1,6]. Importantly, the increase in NE by l-DOPS reached levels higher than that produced by 2 mg/kg of d-amphetamine, the dose used in the previous work reporting the facilitated hemiplegia recovery after sensorimotor cortex ablation [10,12,13].

The purpose of the present investigation was to document the effect of L-DOPS upon beam-walking recovery in rats subjected to unilateral ablation of the right sensorimo-
tor cortex. NE was also assayed in the different regions of the brain in both experimental and control rats to determine whether L-DOPS could possibly lead to an increased tissue level of NE.

2. Materials and method

2.1. Surgery for unilateral cortical lesions

Male adult Sprague-Dawley rats weighing 290–350 g aged 10–13 weeks (SLC, Shizuoka, Japan) were used in the present studies. The animals were individually housed in cages, maintained on a 12:12-h light:dark cycle and given access to food and water ad libitum. The rats were anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg) and mounted in a stereotactic apparatus (Nagashima, Tokyo, Japan). Under aseptic conditions, a square craniotomy was made between 2 mm anterior and 4 mm posterior to the bregma longitudinally, and from 1 mm to 5 mm right of the sagittal suture transversely. The right sensorimotor cortex was exposed at the craniotomy site according to the brain mapping by Neafsey [32] and removed to make a unilateral cortical lesion by gentle aspiration with a fine-tipped Pasteur pipette until the white matter appeared. For hemostasis in the ablation cavity, a small piece of surgical sponge soaked in saline was placed in the cavity, and the scalp was sutured closed.

2.2. Behavioral training and testing

The locomotor deficits resulting from the cortical injury were evaluated on a beam-walking task as described by Feeney et al. [10]. Briefly, prior to surgery the rats were preliminarily trained twice a day for 5 days to traverse an elevated wooden beam 2.5-cm wide and 122-cm long to escape aversive bright light of a 160-W bulb and 62-dB white noise from a tape recorder. At the end of the beam, there was a darkened goal box measuring 25 × 20 × 18 cm. The rats were placed on the beam, and the light and noise were turned on until the animals traversed the beam. All rats were tested at 24 h after surgery, and their beam-walking performances were scored according to the seven-point rating scales described by Feeney et al. [10]. All rats rated as a score of 1 on the pretreatment behavioral test after surgery were then chosen for subsequent postoperative evaluations for functional recovery. These rats with a score of 1 failed to traverse the beam and did not place the left hindlimb on the horizontal surface of the beam. The behavioral measures were made by independent observers who were not informed of treatment conditions.

2.3. Administration of L-DOPS combined with BSZ

The rats with a postoperative score of 1 were then given an i.p. injection of L-DOPS (400 mg/kg) and benserazide (BSZ; 2 mg/kg) dissolved in 0.5% methycellulose solution (n = 10). BSZ, an extracerebral aromatic amino acid decarboxylase inhibitor, was simultaneously injected to block peripheral NE increase and enhance the L-DOPS entering the CNS. Beam-walking performance was consecutively evaluated at 0.5, 1, 2, 3, 6, and 24 h after injection, and every day until the 14th postoperative day. Other groups of rats treated with either 0.5% methycellulose (vehicle) solution (n = 8) or BSZ alone (n = 5) used as a control injection also underwent beam-walking task. Data obtained from the beam-walking task were presented as the mean scores ± standard error of the mean (S.E.M.), and analysed for statistical difference among three groups of rats using Tukey’s nonparametric statistical test. The differences were considered to be statistically significant at the P < 0.05 level.

2.4. Quantitative measurements of NE content in the brain

For biochemical studies of L-DOPS effects on central NE levels, another group of rats were treated either with L-DOPS (400 mg/kg) and BSZ (2 mg/kg) (n = 5) or with the vehicle alone as a control (n = 4) 24 h following the unilateral sensorimotor cortex ablation. These nine rats were sacrificed under deep anesthesia and decapitated 30 min after injection. The brain was removed immediately and dissected on a glass over ice, and the cerebral cortex, hippocampus, and cerebellum of both hemispheres were separately obtained. These specimen were stored at −70°C until quantitative measurements of NE tissue levels using high-pressure liquid chromatography assay [23]. Data were expressed as the means ± S.E.M. Statistical difference on the mean NE tissue levels between the experimental and control groups was assessed by analysis of variance (ANOVA, nested type) for intergroup comparisons with the aid of IBM’s SAS software. P values of 0.05 or less were regarded as statistically significant.

3. Results

3.1. Morphology and histology of the cortical lesion

A representative superior view of the brain following a unilateral ablation injury by aspiration is shown in Fig. 1a. The aspiration lesion was of rectangular shape, 6 × 4 mm in size, with a sharp margin and localized on the right sensorimotor cortex. There was no massive necrosis or hemorrhage in the adjacent brain tissues. The coronal section of the brain demonstrated that the aspiration lesion involved only the cortical layers, not beyond the white matter, and that histological changes were minimal in the adjacent cortical tissues (Fig. 1b).

K. Kikuchi et al. / Brain Research 860 (2000) 130–135
Fig. 1. (a) Representative, macroscopic superior view of the rat brain following a unilateral cortical ablation injury on the right sensorimotor cortex. Bar = 5 mm. (b) Photomicrograph of a coronal section of the brain illustrating the site, extent and depth of the lesion (arrow) (Luxol-fast blue stain, ×40). R = right.
3.2. Behavioral recovery after unilateral cortical injury

The rats treated with a single i.p. injection of 1-DOPS and BSZ demonstrate the most rapid beam-walking recovery among the three animal groups, namely: (a) 1-DOPS and BSZ-treated, (b) BSZ-treated, and (c) vehicle-treated control rats (Fig. 2). At 24 h after injection, the rats given both 1-DOPS and BSZ became capable of traversing the beam and had an average score of greater than 3 in the rating scale. By contrast, the rats given either BSZ or the vehicle failed to traverse the beam and their average rating score was less than 3 during the initial 3 days after injection. During the initial 6 days, the rate of recovery on beam-walking performance was significantly greater in a group of experimental rats treated with 1-DOPS and BSZ than the other two groups [Tukey’s test; (a) vs. (b) \( P < 0.05 \) at 1st, 4th, 5th, and 6th postoperative days, \( P < 0.01 \) at 2nd and 3rd days, (a) vs. (c) \( P < 0.05 \) at 1st, 4th, and 5th days, \( P < 0.01 \) at 2nd, 3rd, 6th, 7th, 8th, and 9th days]. However, the beam-walking performance on the 14th day after injury was not significantly different among three animal groups. In addition, during 10 days after administration it appeared that the behavioral recovery was more impaired in BSZ-treated rats in comparison with vehicle-treated rats. Statistical showed that vehicle-treated rats significantly outnumbered BSZ-treated rats on the beam-walking rating scores at the 6th and 7th days after injection, suggesting that the behavioral recovery was rather hampered in BSZ-treated rats [Tukey’s test; (b) vs.(c) \( P < 0.05 \) at 6th and 7th days]. Therefore, the treatment with 1-DOPS and BSZ was shown to facilitate the rate of motor recovery in a relatively short period of time after cortex ablation.

Fig. 2. The effect of 1-DOPS with BSZ on recovery on beam-walking performance. 1-DOPS was given intraperitoneally together with BSZ 24 h after a unilateral cortical lesion was made. As a control either BSZ or 0.5% methylcellulose (vehicle) solution was administered. Vertical bars indicate S.E.M. scores. Statistical analysis of data using Tukey’s nonparametric statistical test shows that the rate of recovery demonstrated by the animals treated with 1-DOPS and BSZ is significantly different from the other two control groups treated with either BSZ or vehicle during the initial 6 days (* \( P < 0.05 \)). 1-DOPS = L-threo-3,4-dihydroxyphenylserine; BSZ = benserazide; \( n \) = number of animals from which data were taken.

* indicates statistical significance.

3.3. NE tissue levels

The NE levels in the cerebral cortex, hippocampus, and cerebellum of both hemispheres at 30 min after injection were increased in experimental animals given both 1-DOPS and BSZ, and the difference in the NE levels between experimental and vehicle-treated control groups was statistically significant (\( F = 7.56, P < 0.05 \)) (Fig. 3). It was of particular note that more than 10-fold increases in NE levels were produced in the hippocampus after treatment with 1-DOPS and BSZ and the vehicle-treated control group.

4. Discussion

The results of the present studies can be interpreted in several ways: (1) 1-DOPS co-administered with BSZ can facilitate beam-walking recovery at 24 h after their injection following a unilateral ablation of the sensorimotor cortex; (2) 1-DOPS results in a significant increase in the NE tissue level of the brain in the experimental animals. It is postulated that the functional recovery from locomotor deficits produced by 1-DOPS, the precursor of NE, may be related to NE-facilitated recovery, as was previously demonstrated by others in conjunction with an increased release of NE after systemic administration of alpha-2...
antagonists [15,39], catecholamine agonist and amphetamine [8,10,11], or intraventricular injection of NE [4].

L-DOPS, a synthesized precursor of NE, is now clinically used for treating patients with Parkinsonism at the late stage [31]. The precursor crosses the blood-brain barrier and becomes converted into NE in the presence of aromatic amino acid decarboxylase [21,34]. It is demonstrated in the recent in vivo microdialysis studies that L-DOPS concurrently given with a decarboxylase inhibitor (carbidopa) or alpha-2 antagonist results in a significant and prolonged increase in the extracellular NE concentration of the brain [1,6]. MHPG, major metabolite of NE, does not appear to cross the blood-brain barrier [22]. In the current investigation, BSZ, another species of decarboxylase inhibitor, was used at the dose of 1–2 mg/kg, the dose manifesting only minimal effects in the circulating blood. We did not measure the tissue concentrations of MHPG in the present studies. However, our previous data [23] demonstrated that MHPG significantly increased both in the hippocampus and the cerebellum, indicating that the MHPG/NE ratio or NE turnover is increasing in these specific regions. In addition, we examined in the previous investigation [23] the effects of DA, another catecholamine, upon behavioral recovery in the same rodent model subjected to unilateral sensorimotor cortex injury. In contrast to the rats treated with L-DOPS, those treated with L-DOPA (L-3,4-dihydroxy-phenylalanine), which is a precursor for DA, did not demonstrate functional recovery as evaluated by beam-walking performance.

Sequelae resulting from an increase in the NE level in the brain remain to be clarified. There are several behavioral studies elaborating on NE-accelerated recovery on a beam-walking task, but only a few of them discusses the neuroprotective effect of NE against the development of neuronal death [3,9,19]. It is a lesion size and a dosage of catecholaminergic agents used as a “rescue” that determine the degree of postoperative behavioral recovery [16]. Feeney and Hovda [11] reported that catecholaminergic neurons are inhibited following cortical injury, and that the inhibition is alleviated by the treatment with d-amphetamine. One of the underlying mechanisms of this phenomenon is ascribed to a neuroprotective action of NE released by d-amphetamine to ameliorate diffuse inhibition of both metabolism and blood circulation at the region distant from the site of cortical injury. NE may also contribute toward improving cross cerebellar diaschisis [13]. Unlike d-amphetamine, however, beneficial effects of L-DOPS on locomotor recovery persist for a longer period despite a single dose and become further strengthened with subsequent training. Different unknown mechanisms may be involved in this phenomenon.

We have previously documented the effect of chronic intraventricular microinfusion of NE resulted in increased levels of exogenous NE in the brain and significant suppression of delayed neuronal death in the hippocampus of the infused side of the brain in a gerbil model of transient forebrain ischemia [33]. Lee et al. [27] also demonstrated that delayed neuronal death produced by transient forebrain ischemia in gerbils was inhibited by the treatment with L-DOPS and BSZ and suggested that this protective effect of L-DOPS was mediated by the nerve growth factor (NGF) and receptor system. L-DOPS is also reported to increase in vitro NGF synthesis in cultured mouse fibroblasts and astroglial cells [30]. An intraventricular injection of NGF is also capable of ameliorating ischemic hippocampal delayed neuronal death [36]. Activation of the NGF and receptor system should be involved in the survival of neurons after brain injury.

The mechanisms by which amphetamine and other noradrenergic drugs promote beam-walking recovery after sensorimotor cortex injury are speculative. However, several lines of evidence suggest that the recovery of the ability of rats to traverse a narrow beam after unilateral injury to the sensorimotor cortex is noradrenergically mediated [5,18]. As mentioned earlier, amphetamine or NE facilitates recovery from locomotor deficits by alleviating an injury-induced reduction of NE levels and turnover in remote structures. NE is originated from the locus coeruleus, and is widely distributed in the CNS including the cerebral cortex, hippocampus, cerebellum, and spinal cord. Intra-hippocampal injection of amphetamine elevated the regional NE level and enhanced memory retention in rats [26]. Krobert et al. [25] documented amphetamine-induced significant increase of cerebellar NE in both uninjured controls and contused rats. Beam-walking performance is an integrated form of behavior necessitating pertinent levels of consciousness, memory, sensorimotor and cerebellovestibular functions. Amphetamine-accelerated recovery involves not only locomotor function but also sensory and visual function, vestibulocerebellar function, as well as learning and memory. It is therefore implicated that NE is one of the nonspecific neuromodulators favorably influencing functional recovery.

In view of the previous studies documenting amphetamine-facilitated restoration of locomotor function both in the animal model of sensorimotor cortex ablation [2,12] and in hemiplegic patients with cerebral infarction [8,29], our current data highly support a significant role of NE in the beam-walking recovery after cortical injury, and further indicate that L-DOPS could become a promising pharmacotherapeutic candidate for parallel experiments with d-amphetamine and hemiplegic stroke patients.

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