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Intraventricular Norepinephrine Facilitates Motor Recovery Following Sensorimotor Cortex Injury

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BOYESON, M. G. AND D. M. FEENEY. *Intraventricular norepinephrine facilitates motor recovery following sensorimotor cortex injury.* PHARMACOL BIOCHEM BEHAV 35(3) 497–501, 1990.—Intraventricular norepinephrine, dopamine, or vehicle was administered to rats 24 hours after a unilateral sensorimotor cortex ablation to determine their potential roles in acceleration of motor recovery as measured by the beam-walking task. Norepinephrine was found to be the critical neurotransmitter in facilitating motor recovery. Blocking norepinephrine synthesis by dopamine-β-hydroxylase inhibition coupled with dopamine administration failed to accelerate recovery, indicating a more important role for norepinephrine compared to its precursor dopamine in motor recovery after sensorimotor cortex injury.

Norepinephrine Brain injury Sensorimotor cortex Recovery of function Dopamine

RELATIVE to other proposed mechanisms or theories of recovery of function after brain injury, diaschisis has received considerably less attention, perhaps in part due to a lack of any hypothetical processes specifically operating in the remission of symptoms. Diaschisis, formulated from clinical observations by von Monakow (29), is a hypothetical depression of neuronal function in an area remote from, but connected to, a primary site of injury (14,16). According to this theory, some recovery of function will occur as the remote functional depression dissipates. Older experimental studies of diaschisis (18,23), while suggestive, have not provided conclusive support for the concept [see review by Feeney and Baron (14,24)]. Only a few recent studies (13, 21, 26) have correlated a behavioral change with a depression of metabolic function in a structure remote from an injury, and have shown that both measures respond in parallel to pharmacotherapy. As has been suggested (27), if such manipulations of neurotransmitter systems parallel changes in recovery from symptoms following brain injury, dissipation of diaschisis could be entertained as a viable mechanism of recovery.

Recently Feeney and Hovda (10) have proposed that catecholamine (CA) systems are depressed after cortical injury and AMP treatment alleviates this depression. They suggest that a "CA diaschisis" occurs following some brain injuries. Evidence for the involvement of the CAs comes from observations that haloperidol, a CA antagonist (6), retards recovery of beam-walking (11), while amphetamine (AMP) and other CA agonists accelerate recovery [see Feeney and Sutton (15) for review].

The present study was undertaken to clarify the role of the CAs (norepinephrine, NE; dopamine, DA) in the effect of AMP on recovery of locomotion after unilateral sensorimotor cortex ablation. To determine if NE or DA is involved in the acceleration of recovery of AMP after sensorimotor cortex ablation, intraventricular infusions of NE or DA were investigated in a rat model of hemiplegia. To further discriminate the relative contributions of each catechol (DA or NE) to beam-walking recovery processes, the enzyme converting DA to NE was inhibited in one group and, in the presence of the inhibitor, additional DA was added to bias recovery toward DA in another group. These data have been partially published in a preliminary form (4).

METHOD

Subjects

A total of 56 male Sprague-Dawley rats (Harlan-Gibco) weighing 300–324 g at the beginning of the experiment were used as subjects. The rats were individually housed and fed ad lib in a temperature-controlled (22°C) room on a 12-hr light/dark cycle.

Apparatus

The apparatus to assess beam-walking ability was a long (122 cm), narrow (2.5 cm), elevated (36 cm) beam. A speaker placed at the start position broadcast a loud (62 dB) tape-recorded white

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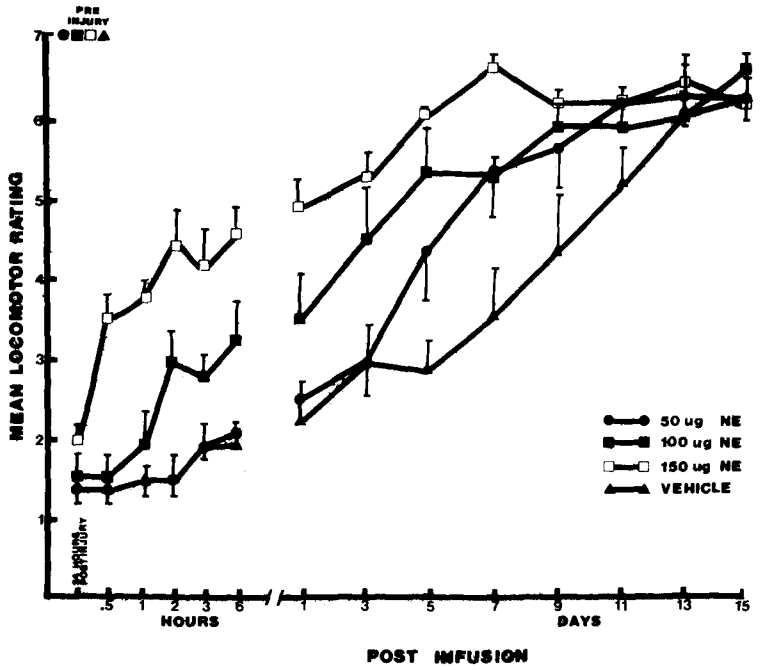

FIG. 1. The above graph depicts the effect on the beam-walk task of intraventricular infusions of norepinephrine (NE) or vehicle on locomotor ability following unilateral sensorimotor cortex ablation. Statistical analyses indicated that only 100 μg/10 μl and 150 μg/10 μl of NE significantly accelerated recovery of locomotor ability on the beam walking task (see text for details).

noise, which was terminated when the animal entered a large, dark goal box (24.8 × 20.3 × 17.8 cm) at the end of the beam.

Procedure

Training on the beam-walk task began after a one-week habituation period. On the first training day, the animal was given three trials. On trial one the animal was placed just outside the goal box; on trial two, at the midpoint of the beam; and on trial three, at the start position. Single trials were then conducted every other day and the animals rated on a seven-point scale by two observers, one "blind" to treatment conditions. Prodding was used if the rat stopped or hesitated in running on the beam (19). The rating scale is described in detail elsewhere (11,15) and with parameters affecting recovery outcomes (19). In brief, if animals traversed the beam with no more than two hindlimb footslips, they were rated the maximum score of "7." If animals obtained a "7" on two successive trials (usually within 2–4 trials), no further training was conducted so that slower animals were able to attain the same presurgery performance level, avoiding "overtraining" some animals. The day prior to surgery all animals were given a single test trial to insure continued performance at a "7" level. Animals not maintaining this level were dropped from the study. Postsurgical testing times for all experiments began at 1100–1200 hr.

For surgery the animals were anesthetized with pentobarbital sodium (Nembutal; 21 mg/kg, IP) preceded by ketamine hydrochloride (Ketacet; 60 mg/kg, IM) and placed in small animal stereotactic apparatus (David Kopf Instruments). A craniotomy was performed over the right sensorimotor cortex, the dura was excised, and the animal received a right unilateral sensorimotor cortex ablation (by gentle aspiration using a vacuum pump) extending from 2 mm anterior and 4 mm posterior to bregma, and 5 mm lateral from the sagittal sinus to a depth of the white matter. The cavity was filled with sterile gelfoam.

A small burr hole was drilled through the cranium of the left hemisphere over the lateral ventricle for the later infusion (at 24 hr postinjury) of either NE, DA, or vehicle control (0.1% ascorbic acid in sterile physiological saline). The scalp was then sutured closed.

In the first study four groups of animals (N = 8 each) were trained on the beam-walking task. The first three groups were either given 50 μg/10 μl NE, 100 μg/10 μl NE or 150 μg/10 μl NE [a dose range utilized in a memory retrieval task (1)]. The fourth group was given the vehicle solution. In the second study, four groups of animals (N = 8 each) were trained on the beam-walking task. In group one, the animals received 150 μg/10 μl DA [two lower doses (50 and 100 μg) were found to be ineffective in pilot studies]; in group two, animals received 150 μg/10 μl DA plus FLA-57 (4-methyl-1-homopiperazinedithiocarboxylic acid) 30 mg/kg (2,17); group three received only FLA-57; and group four received the vehicle infusion. FLA-57, rather than FLA-63, was chosen on the basis of its effectiveness in enzyme inhibition [through copper chelation; (2)] and lack of severe side effects (17). The enzyme inhibitor was administered 4 hours prior to DA infusion, when inhibition of NE is maximal (2,17).

At 23 hr postinjury, immediately after the baseline postsurgery beam-walk task, the animal was lightly anesthetized with ether, placed in a stereotactic holder, and the suture reopened. A microliter injection syringe mounted on the stereotactic holder and containing the drug was inserted through the burr hole and into the left lateral ventricle contralateral to the right hemisphere ablation, and the drug or vehicle was slowly infused (manually, approximately 2.5 μl every 30 sec) over a 2-min period. After the drug infusion, the animal was returned to his home cage and tested on the beam-walking task at 0.5, 1, 2, 3, 6, and 24 hr, and then every other day until day 15 postinjury. At the end of the experiment, all

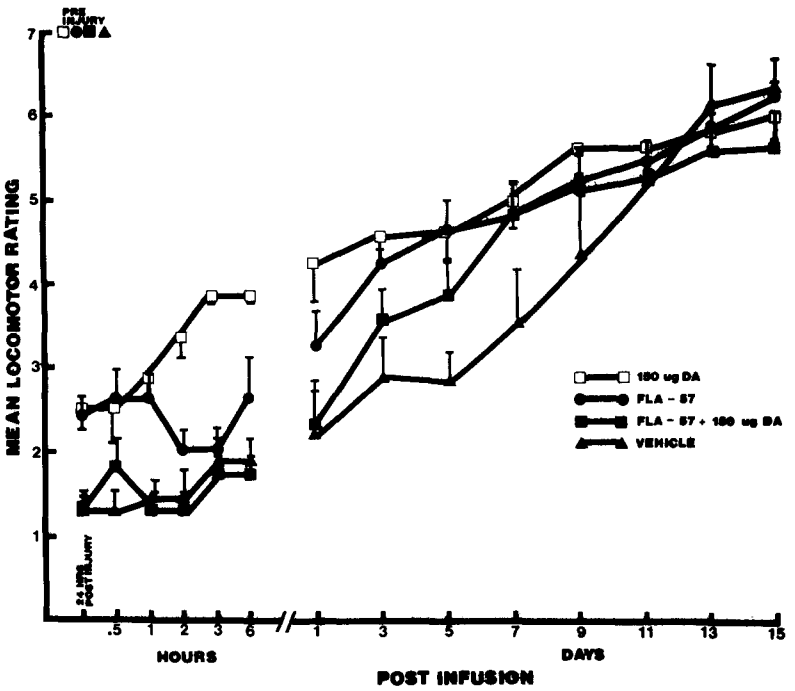

FIG. 2. The above graph depicts the effect of blocking norepinephrine (NE) synthesis and concomitant dopamine (DA) administration on the beam-walking task. Blocking of NE synthesis by the dopamine-β-hydroxylase inhibitor FLA-57 (30 mg/kg) in combination with 150 μg DA failed to accelerate recovery of locomotor function following SMCX injury (see text for details).

animals were sacrificed with an overdose of pentobarbital sodium. The animals were perfused through the heart with 0.9% saline followed by a 10% formalin solution. The brains were removed and 40-μ sections were taken using a cryocut microtome to verify lesion and ventricular insert location. All behavioral data obtained from beam-walking observations were analyzed using a repeated measures analysis of variance. Comparisons were made between groups on rates of recovery following lesions and drug treatments. Interrater reliability between different observers was high on the beam-walking assessment as no score differences were noted.

RESULTS

The results obtained from infusions of NE and vehicle control are presented in Fig. 1. Twenty-four hours after surgery and before intraventricular infusion of the drugs, all animals were severely debilitated on the beam-walking task, but all groups recovered mobility on the task by 15 days postdrug. Both the 100 and 150 μg doses of NE significantly, $F(1,15)=5.82$, $p<0.03$; $F(1,15)=8.71$, $p<0.01$, facilitated recovery of function compared to vehicle controls.

Although the 150 μg dose of DA did not achieve significance, it was close enough to give us concern that perhaps DA was partially involved in the recovery of beam-walking. The possibility existed that DA could be taken up by NE cells and, therefore, be affecting recovery indirectly through the NE system. To assess this possibility, animals were given FLA-57 with DA or alone. Clearly, as represented in Fig. 2, when DA was given in combination with the enzyme inhibitor (preventing conversion to NE), no facilitated effect on recovery was observed, suggesting that NE, rather than DA, was the critical catechol in facilitating beam-walking recovery. Although it appeared that DA was facilitating recovery during the first 6 hours after administration, this was due to the fact that two groups were significantly higher in recovery in the first postinjury assessment period than the vehicle group. (An ANCOVA with repeated measures subsequently run on this 6-hour period, controlling for the difference in recovery levels between vehicle and the DA group, was insignificant.)

The average volume of cavitation induced by aspiration injury for each group (intended volume 75 mm^3) was calculated by multiplying the square areas of individual sections by the distance between sections, and summing the cubed values through the AP extent of the lesion. If an animal showed evidence of direct lesion to caudate (or other subcortical areas), the animal was dropped from analysis (two animals were dropped). Verification of syringe insertion into the ventricle was defined as a breach of the lateral ventricular wall without evidence of passing completely through the ventricle (all animals used fit the criteria). The following values were obtained for each group's volume (in parentheses), with the maximum and minimum values of the AP extent following for the largest and smallest lesions according to the coordinates of Paxinos and Watson (25). No significant differences were found between groups for the volume of the lesion. The largest AP extent with reference to bregma of the 150 μg NE group (71.7 ± 3.6 mm^3) was $+2.7\rightarrow-3.8$, the smallest was $+2.2\rightarrow-3.3$; in the 100 μg NE group (73 ± 1.1 mm^3) the largest was $+2.8\rightarrow-3.5$, the smallest was $+2.4\rightarrow-3.5$; in the 50 μg NE group (72.1 ± 2.8 mm^3) the largest was $+2.7\rightarrow-3.5$, the smallest was $+2.2\rightarrow-3.3$; the vehicle group (75.9 ± 3.08 mm^3) largest AP extent was $+2.8\rightarrow-3.3$, the smallest $+2.4\rightarrow-3.3$; the 150 μg DA group (73.9 ± 3.5 mm^3) largest AP extent was $+2.8\rightarrow-3.3$, the smallest $+2.2\rightarrow-3.5$; the 150 μg DA +FLA-57 group (68.27 ± 3.1 mm^3) largest AP extent was $+2.9\rightarrow-3.3$, the smallest $+2.7\rightarrow-3.1$; the FLA-57 group (68.4 ± 2.9 mm^3) largest AP extent was $+2.7\rightarrow-3.6$, the smallest was $+2.2\rightarrow-3.3$.

DISCUSSION

Due to the rapid onset of the effect on recovery, the NE data are most likely a result of direct postsynaptic actions. This acceleration of recovery is quite similar (within 1–2 hr) to that induced by a single dose of AMP given 24 hr postsurgery (11,15). The results suggest that the previous findings of an immediate AMP induced acceleration of recovery on a beam-walking task following sensorimotor cortex ablation in rat or cat (11,20) are due to AMP's effect on NE rather than DA. In support of this, our previous research has indicated that NE blockers reinstate deficits in recovered animals (4,15), providing further support for the NE hypothesis. Nevertheless, it is not entirely clear why haloperidol retards recovery, although it could be due to haloperidol's potent blocking of alpha-1 receptors (6), instead of its better known receptor blocking actions on the dopamine system. Interestingly, clonidine and phenoxybenzamine both are more effective in reinstating deficits in recovered animals than is haloperidol (4,15). Clonidine would be inhibiting locus ceruleus activity (via presynaptic inhibition) while phenoxybenzamine would act by blocking alpha-1 receptors (similar to haloperidol's actions).

The NE projections to cortex are interrupted by SMCX injury, and it could be the case that a transient disruption in NE functioning elsewhere, e.g., in the cerebellum (4, 5, 8), may be responsible for the hemiparesis rather than a lesion of the SMCX area per se. Our preliminary pilot work has indicated that local microinfusions of NE into the contralateral, but not ipsilateral cerebellum to the SMCX injury rapidly facilitate recovery from the hemiparesis (5), indicating the strength for a transient depression in NE functioning hypothesis.

In a generally similar fashion, NE has been implicated in visual system plasticity, and it has been suggested that recovery from injury may be a subset of a generalized neural plasticity. Kasamatsu *et al.* (22) have reported that intracortically infused NE can restore binocularly driven visual cortex neurons following monocular deprivation but this work has some controversy (3,28). However, similar to previous work on AMP-induced recovery of beam-walking (11,20), NE alone is not sufficient since "experience" during the period of drug action is required for the acceleration of functional recovery. Consistent with the important role of experience (11), AMP restores stereopsis in adult cats with bilateral lesions of visual cortex only if the cats are given visual experience (12).

The implications of the present study suggest that noradrenergic drugs, perhaps the tricyclic antidepressants, may have some benefit on functional recovery if relevant therapy is given while under the effect of the drug. In stroke patients, AMP administration coupled with physical therapy has been shown to benefit motor recovery (7,9). Understanding the mechanisms involved in this potential pharmacological treatment (e.g., effect on learning/memory, performance variables) could provide the basis for optimizing therapy for an otherwise untreatable condition.

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