Effects of $d$-Amphetamine on the Recovery of Function Following Cerebral Ischemic Injury

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COULBOURNE, F. AND D. CORBETT. Effects of $d$-amphetamine on the recovery of function following cerebral ischemic injury. PHARMACOL BIOCHEM BEHAV 42(4) 705–710, 1992.—Amphetamine with appropriate motor experience has been found to facilitate the recovery of motor function after several different types of brain injuries. We investigated whether amphetamine would hasten the recovery of spatial mapping ability in gerbils previously subjected to a 3-min episode of forebrain ischemia. Amphetamine did not promote behavioral recovery, nor did it attenuate ischemic cell damage of hippocampal CA1 neurons. The beneficial effects of amphetamine after brain injury may be limited to restoration of sensorimotor ability and not to cognitive functions such as memory.

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<th>Ischemia</th>
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DIASCHISIS, originally proposed by von Monakow (19), is a theory of recovery of function following brain injury. According to this view, behavioral impairments are due, in part, to a “functional depression” remote from the site of injury. Thus, any treatment removing this remote depression would accelerate functional recovery after a brain insult. Feeney and colleagues (9) were the first to show that amphetamine could hasten recovery of beam-walking behavior after a sensorimotor cortex lesion in rats. Moreover, a single dose of amphetamine given before one beam-walking task accelerates subsequent recovery of motor function after frontal and sensorimotor cortex lesions (9,13,14). Crisostomo and colleagues (7) have also shown that amphetamine with physical therapy promotes motor recovery in stroke patients. Since amphetamine increases release of catecholamines (CA), a CA diaischisis has been proposed (10). Recent evidence suggests that norepinephrine (NE), and not dopamine (DA), is the critical transmitter (4).

Brief periods of global ischemia in the gerbil produce profound hippocampal CA1 cell loss (15,16). This type of lesion results in a variety of deficits involving spatial navigation (2), working memory (8), and passive avoidance behavior (1). Open-field activity in a novel environment increases dramatically following an ischemic insult. This increased activity does not reflect a simple form of motor hyperactivity since preexposure to the open field prior to ischemia blocks the development of increased open field activity. Instead, ischemic injury to CA1 may impair an animal’s ability to form spatial maps or habituate to novel environments (20). The present study assessed whether amphetamine when administered prior to open-field testing could accelerate recovery of spatial mapping in gerbils previously subjected to an ischemic insult.

GENERAL METHOD

Subjects

Sixty female Mongolian gerbils (High Oak Ranch Ltd., Goodwood, Ontario, Canada), weighing between 50–100 g at the time of surgery, were used in Experiment 1. Ten gerbils were used in the second experiment. Animals were housed separately in plastic cages under diurnal light conditions. Both food and water were available ad lib.

Procedure

Gerbils were anesthetized with 2% halothane, and maintained with 1.5% halothane, in a mixture of 30% oxygen and 70% nitrous oxide. The common carotid arteries were isolated and then occluded with microarterial clamps (Fine Science Tools, Vancouver, British Columbia, Canada) for 3 min. In sham-operated animals, the carotid arteries were exposed but not occluded.

During surgery, both body and skull temperature were maintained between 37–38°C using a homeothermic blanket unit (Harvard Apparatus, South Natick, MA) and a heated water flow-through blanket system wrapped partially around the gerbil’s head. Skull temperature, both during and after surgery, was measured by a 30-ga thermocouple probe in-
serted subcutaneously into the skull (Omega Engineering, Stanford, CT). Skull temperature was monitored for 15 min following the completion of surgery (i.e., approximately 20 min postocclusion). Longer recording times were not possible since most animals would begin to show signs of discomfort after 15–20 min. Rectal temperatures were taken usually at .5, 1, 2, and 3 h after surgery (data not shown). Rectal temperatures were not recorded after surgery in the second experiment.

All gerbils were tested in the open field at 24 h, 7, and 10 days following surgery for 10 min each day. The open field measured 75 x 75 x 58 cm and was divided into 25 equal squares. An image tracking system (HVS Systems, Kingston, UK) recorded the total number of squares the gerbil crossed in each 10-min trial.

EXPERIMENT 1

METHOD

The saline-treated group (SALINE) in Experiment 1 received a 0.1-ml IP injection just after suturing while still under anesthetic. The sham-operated group (SHAM) received 1.0-mg/kg IP amphetamine at a similar time as the SALINE group. The 1.0-mg/kg IP (LOW) and 2.0-mg/kg IP (HIGH) amphetamine groups received their injections also at this time. The 24-h pre-open-field group (PRE) received 1.0 mg/kg IP 15 min prior to the start of the first open-field test (i.e., approximately 24 h after surgery). The 24-h post-open-field group (POST) received 1.0 mg/kg IPamphetamine just after completion of the first open-field test.

Animals were killed either 10 or 11 days after surgery with an overdose of sodium pentobarbital. They were then transcardially perfused with 30 ml 0.9% heparinized saline followed by 30 ml 10% phosphate-buffered formalin. Brains were stored in buffered formalin for at least 10 days. Brains were transferred to a solution of 20% sucrose in 10% phosphate-buffered formalin 1–2 days prior to sectioning. Tissue was sliced at 10 µm and stained with cresyl violet.

CA1 pyramidal cells were examined in three equal sectors corresponding to medial, middle, and lateral portions of the CA1 pyramidal cell layer at a level approximately 1.7 mm posterior to Bregma (18). Each sector was rated on a five-point scale as follows: 90–100% normal cells = 4; 60–89% = 3; 30–59% = 2; 6–29% = 1; and 0–5% = 0 (Fig. 1). These three sectors were next to subiculum (medial), at the apex of CA1 (middle), and next to CA3 (lateral). The rating scores from all six (three per hemisphere) sectors were added

![FIG. 1. Photomicrographs illustrating examples of the CA1 rating scores. From the top: 4 = 90–100% of cells are normal; 2 = 30–59% of cells are normal, and 0 = less than 5% of cells are intact. Cresyl violet stain, bar = 150 µm.](image)

![FIG. 2. CA1 cell ratings (mean ± SD) for each of the groups in Experiment 1. Medial, middle, and lateral sectors of CA1 from both hemispheres (2 x 3) were rated on the five-point scale described in Fig. 1. See the General Methods section for further details.](image)
to yield a cumulative CA1 cell rating with a maximum score of 24 (i.e., a normal CA1). Two investigators blinded to the treatment conditions performed the ratings.

Analysis of variance (ANOVA), Student's t-tests, and Fisher's protected least-significant difference (LSD) were used to analyze the day 7 open-field activity levels for Experiment 1. Histological results were analyzed with Kruskal-Wallis and Mann-Whitney U-tests.

RESULTS

All groups (i.e., SALINE, LOW, HIGH, PRE, and POST) had significantly lower cell ratings (Fig. 2) than SHAM animals, $H = 21.164, p < 0.05$, $U = 0, p < 0.005$, $U = 0, p < 0.005$, $U = 7.5, p < 0.005$, $U = 2, p < 0.005$, and $U = 3.5, p < 0.005$, respectively. Gerbils treated with amphetamine just prior to the first open-field test (i.e., PRE group) showed some attenuation of CA1 cell necrosis compared to POST and SALINE animals; however, this difference did not attain statistical significance, $U = 31, p > 0.05$, $U = 22.5, p > 0.05$, respectively (Fig. 2). This group also displayed an accelerated habituation to the open-field test on day 7 compared to SALINE and POST animals, $t(17) = 2.34, p < 0.025$, and $t(16) = 3.37, p < 0.025$, respectively (Fig. 2). The PRE and POST gerbils had similar skull temperatures during surgery (Fig. 4). However, PRE animals did not exhibit the postischemic rise in temperature that typified the other groups.

Amphetamine administered early after ischemia (LOW and HIGH) did not affect histological outcome as compared to SALINE animals, $U = 38.5, p > 0.05$, $U = 43.5, p > 0.05$, respectively. These LOW and HIGH groups were also not functionally different on day 7 than SALINE animals, $F(5, 53) = 5.84, p < 0.001, p > 0.79$, and $p > 0.19$, respectively (Fisher's protected LSD posthoc test).

DISCUSSION

Amphetamine given 24 h after a brief period of ischemia and prior to spatial mapping experience appeared to accelerate functional recovery of spatial mapping ability. However, the histological protection seen in the PRE group is not easily explained since gerbils receiving amphetamine just after the open field test (i.e., POST group) were not protected. Also, it would be surprising if amphetamine administered 24 h after ischemia provided histological protection since the cascade of events leading to ischemic cell death begin in the first few hours of reperfusion (15, 16). The protection seen in the PRE group may instead be due to an attenuation of postischemic hyperthermia in this group compared to SALINE and POST groups. This lack of postischemic hyperthermia could have afforded PRE gerbils both histological and functional protection since postischemic hyperthermia aggravates ischemic damage (17). Therefore, Experiment 2 was carried out to determine if amphetamine would have any beneficial effect in animals that have similar postischemic temperatures.

EXPERIMENT 2

METHOD

Saline-treated gerbils (SALINE-2, $n = 4$) received 0.1 ml saline IP 15 min prior to the first open-field test. A pre-open field group (PRE-2, $n = 6$) received 1.0 mg/kg IP amphetamine 15 min prior to the first open-field test, as in Experiment 1.
FIG. 4. Skull temperatures (Experiment 1) recorded in animals at the start of surgery (T1), the start of occlusion (T2), the end of occlusion (T3), the end of surgery (T4), and at intervals (min) following surgery.

FIG. 5. Experiment 2 open-field activity scores (mean) per each 10-min test session on postischemia days 1, 7, and 10.
FIG. 6. Skull temperatures (Experiment 2) recorded in animals at the start of surgery (T1), the start of occlusion (T2), the end of occlusion (T3), the end of surgery (T4), and at intervals (min) following surgery.

ment 1. Unlike Experiment 1, gerbils were selected and assigned to groups on the basis of their skull temperatures at the end of surgery before injections were given. This was to ensure that both groups had comparable skull temperatures. All other details concerning behavioral testing, surgery, and histological analysis were as described for Experiment 1.

RESULTS

There were no differences in histological outcome or in open field activity (Fig. 5) between SALINE-2 and PRE-2 gerbils. These groups had a mean cell rating of 4.25 (2.49, SD) and 4.83 (2.48, SD), respectively. Moreover, these two groups had similar skull temperatures during surgery and in the early postischemic period (Fig. 6).

GENERAL DISCUSSION

In Experiment 2, amphetamine, with spatial mapping experience (PRE-2), did not accelerate functional recovery in gerbils previously subjected to a brief period of global ischemia. It also did not provide histological protection. Therefore, any protection in the PRE group seen in Experiment 1 appears to be due to a reduction in postischemic hyperthermia. While it is not clear why the PRE group in Experiment 1 failed to exhibit postischemic hyperthermia, it does emphasize the importance of postischemic temperature in histological and functional outcome.

The results also suggest that amphetamine does not alter CA1 cell death when given in a single intraperitoneal dose either immediately or delayed 24 h after ischemia. Amphetamine increases release of both NE and DA. Elevated NE levels are believed to reduce (3,12), while increased DA levels may worsen ischemic damage (5,6,11). In the present experiment, amphetamine's effects on NE and DA could have cancelled each other out, thus resulting in no histological effect. However, recent data from our laboratory (Nurse, Evans, and Corbett, submitted) suggest that DA does not play an important role in CA1 ischemic injury.

Amphetamine's ability to accelerate functional recovery may be limited to motor and not cognitive functions. Amphetamine, by itself, does not alter histological outcome of CA1. However, increased DA release has been associated with striatal injury (11). Thus, stroke treatments, such as physical therapy, involving amphetamine administration soon after a stroke might have histological consequences. Therefore, it might be preferable to use more selective pharmacological agents such as idazoxan that elevate levels of NE but not DA. It would also be valuable to test if amphetamine, or more selective NE-elevating agents, could accelerate performance on other behavioral tests (e.g., T-maze, passive avoidance) after an ischemic insult. Finally, one must be careful that amphetamine and related compounds do not cause hyperthermia, which might subsequently worsen outcome. In conclusion, removal of diaschisis or remote functional depression by amphetamine and related compounds has limited but potentially useful benefit in stroke treatment.

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REFERENCES


