Lateralized Effect of Cerebral Infarction on Spinal Fluid Monoamine Metabolite Concentrations in Rats

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Using a rat model of stroke, we studied the effect of unilateral middle cerebral artery ligation on cerebrospinal fluid monoamine metabolites at different intervals over a 40-day postoperative period. Male Sprague-Dawley rats were divided into four groups: an unoperated control group (n=9), a sham-operated group (n=9), a right middle cerebral artery ligation group (n=10), and a left middle cerebral artery ligation group (n=10). One hundred microliters of cerebrospinal fluid were collected percutaneously from the cerebellomedullary cistern just before and 5, 20, and 40 days after the surgical procedure. Monoamine metabolites — 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) — were measured using high-performance liquid chromatography. MHPG concentration in the right lesion group was significantly depleted from control levels 5, 20, and 40 days after surgery. No such depletion was observed in the left lesion rats. Concentration of 5-HIAA was relatively lower at Days 5 and 20 in the right lesion group than in the left lesion group. HVA concentration did not differ among the groups at any time. Our study has demonstrated a differential effect of unilateral ischemia on cerebrospinal fluid neurochemistry in rats dependent on the cerebral hemisphere involved. (Stroke 1988; 19:472-475)

In previous studies we have demonstrated that the neurochemical consequences of right middle cerebral artery (MCA) ligation in rats include a 30-40% depletion in norepinephrine concentrations in the ipsilateral cortex and the ipsilateral and contralateral brainstem and a 65-70% depletion of dopamine concentrations bilaterally in the substantia nigra, with partial or complete recovery of the concentration of both catecholamines during a 40-day postoperative period. In addition, this biochemical response to experimental stroke was lateralized. Right MCA ligation produced significantly greater depletions of catecholamines than comparable lesions of the left hemisphere. Other studies, too, have demonstrated the effect of cerebral ischemia or focal cortical injury in rats on brain biogenic amine concentrations.

Our animal model of stroke has complemented our investigations into the etiopathogenesis and mechanisms of poststroke depressive disorders in humans. For instance, our studies of stroke in humans have also found lateralized effects of cerebral ischemia on mood: acute left frontal stroke has been associated with major depression, whereas right frontal lesions lead to undue cheerfulness and loss of interest. We have hypothesized that lateralized biochemical responses to stroke in humans may underlie the lateralized mood changes. If these mood changes are to be related to neurochemical changes, however, it is important to show parallels between the neurochemical consequences of lesions in humans and rats. Studies by previous investigators have suggested that stroke in humans affects cerebrospinal fluid (CSF) catecholamine metabolites and that the effect differs depending on the hemisphere injured. Our present study examines the effect of unilateral MCA ligation on concentration of CSF monoamine metabolites in rats and follows these concentrations over a 40-day postoperative period.

Materials and Methods

Male Sprague-Dawley rats weighing approximately 250-275 g were caged individually with food and water available ad libitum for 3 weeks before surgery. The cages consisted of a stationary wire mesh compartment 8 × 13 × 28 cm and a 34-cm diameter running wheel that rotated freely in either direction. Rats were maintained on a 12:12 hour light:dark cycle.

Surgery

Under 3 ml/kg i.p. chloropent anesthesia, rats were placed in a stereotactic apparatus and a frontoparietal craniotomy was performed. The craniotomy site extended from just anterior to the coronal suture posteriorly to the periorbital area anteriorly and from the zygomatic arch inferiorly to just below the ridge between the dorsal and lateral skull superiorly. The MCA was ligated as inferiorly in this craniotomy site as was technically feasible. Under a dissecting micro-
scope, a 6-0 ophthalmic suture was passed through the dura behind the MCA and out through the dura again. The MCA and overlying dura were ligated, and the MCA was severed distal to the tie using microscissors. The temporal muscle was flapped over the craniotomy site. Rats were randomly selected for ligation of either the right or left MCA. The surgical approach on both sides was identical, and there appeared to be no gross anatomic differences between the right and left MCA. Sham operations consisted of performing the craniotomy on either the right or left side without ligating the MCA. We also included a group of unoperated control rats. The postoperative mortality associated with the procedure was 4 of 42 rats. The postoperative morbidity, in terms of obvious discomfort to the rats, appeared to be negligible based on their rapid return to preoperative levels of food intake and activity within a couple of days after surgery.

Collection of Cerebrospinal Fluid

One hundred microliters of CSF were collected by a percutaneous technique from the cerebellomedullary cistern under ether (n = 34) or chloral hydrate (n = 4) anesthesia just before MCA ligation as well as 5, 20, and 40 days after surgery.

Rats were restricted from access to the running wheel during the night before CSF collection to minimize the effects of activity on concentrations of the monoamine metabolites. CSF collections were done between 10 AM and noon and, for the same rat, within 5 minutes from one sample day to the next to minimize any effect of diurnal variation in these metabolites.

The CSF monoamine metabolites free 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) were measured using high-performance liquid chromatography, a modification of the assay described by Zaczek and Coyle. In our assay, the mobile phase was 13.6 g sodium acetate, 2.0 g heptane sulfonate, and 60 ml acetonitrile in 1,940 ml distilled water at pH 3.2. The liquid chromatographic system consisted of an Altex 110 pump (Alltech Associates, Inc., Deerfield, Illinois), a Rheodyne 7120 injector (Cotati, California), a Brownlee Laboratories RP 18 46 x 100 mm column (Santa Clara, California), and an ESA, Inc. 5100A electrochemical detector (Bedford, Massachusetts). The eluting flow rate was adjusted to 1.5 ml/min, and 20 μl of each CSF sample was injected on the separation system after centrifugation. The electrochemical detection system consisted of an ESA 5011 analytical cell and an ESA 5021 guard cell with an ESA 5100A electrochemical detector. The monoamine metabolites were identified and quantified with a Hewlett-Packard Co. 3390 A detector (Palo Alto, California) by comparing their retention times (3.2, 14.3, and 18.8 minutes for MHPG, 5-HIAA, and HVA, respectively) and their peak heights with fresh mixtures containing an internal standard (3,4-dihydroxybenzylamine, retention time 14.4 minutes).

Data Analysis

Each metabolite was analyzed separately using repeated-measures analysis of variance (ANOVA). Individual differences were determined using planned analyses t tests, which used a pooled error term.

Results

Concentrations of all three CSF monoamine metabolites were not significantly different between the sham-operated (n = 9) and the unoperated (n = 9) controls at any time. Hence, these two groups were combined into a single control group (n = 18) for comparison with the right MCA ligation (n = 10) and left MCA ligation (n = 10) rats.

There was a significant group × time interaction (F 6105 = 2.82, p < 0.05) for MHPG. A planned comparison analysis revealed significant reduction in MHPG concentrations in the right MCA ligation group compared with the control group 5 (p < 0.05), 20 (p < 0.01), and 40 (p < 0.01) days after surgery and a trend toward reduced MHPG concentration in the right MCA ligation rats compared with left MCA ligation rats 5 (p < 0.1) and 20 (p < 0.1) days after surgery (Table 1, Figure 1). No differences were seen between the left MCA ligation and control groups.

Repeated-measures ANOVA showed a significant group × time interaction (F 6105 = 3.11, p < 0.01) for 5-HIAA. A planned comparison analysis attributed the source of the differences to lower 5-HIAA concentrations in the right than the left MCA ligation groups 5 (p < 0.05), 20 (p < 0.01), and 40 (p < 0.01) days after surgery (Table 1). There was a similar trend when comparing the right MCA ligation with the control rats on Day 5 (p < 0.1), while there were no differences between the left MCA ligation and control rats at any time (Table 1, Figure 1).

| Table 1. Cerebrospinal Fluid Monoamine Metabolites in Rats With or Without Middle Cerebral Artery Ligation |
|--------------------------------------------------|--------------------------------------------------|----------------------------------|
| Control (n = 18) | Left (n = 10) | Right (n = 10) |
| 3-Methoxy-4-hydroxyphenylglycol | | |
| Preoperative | 18.1 ± 1.1 | 17.5 ± 2.4 | 19.1 ± 1.4 |
| Day 5 | 18.8 ± 2.0 | 18.4 ± 1.9 | 13.8 ± 1.2^* |
| Day 20 | 19.3 ± 1.3 | 17.2 ± 1.2 | 12.7 ± 1.2^† |
| Day 40 | 18.3 ± 1.4 | 15.8 ± 1.8 | 12.8 ± 1.4^† |
| 5-Hydroxyindoleacetic acid | | |
| Preoperative | 69.6 ± 5.0 | 72.5 ± 5.6 | 77.9 ± 7.7 |
| Day 5 | 88.1 ± 5.9 | 99.4 ± 6.6 | 72.0 ± 5.5^‡ |
| Day 20 | 79.4 ± 2.8 | 91.6 ± 8.1 | 66.2 ± 4.4 |
| Day 40 | 85.4 ± 6.4 | 77.8 ± 5.5 | 77.8 ± 6.9 |
| Homovanillic acid | | |
| Preoperative | 11.8 ± 1.1 | 12.5 ± 1.1 | 13.2 ± 2.2 |
| Day 5 | 14.1 ± 2.1 | 14.3 ± 2.3 | 10.5 ± 1.7 |
| Day 20 | 11.7 ± 1.1 | 13.8 ± 1.4 | 9.0 ± 0.5 |
| Day 40 | 14.2 ± 1.0 | 13.6 ± 1.3 | 14.1 ± 1.7 |

Values are mean ± SEM ng/ml.
^*p < 0.05 different from control.
†p < 0.01 different from control.
‡p < 0.01 different from left ligation.
There was no significant group \times time interaction ($F_{6,105} = 1.54, p > 0.1$) for HVA. Thus, no significant changes in HVA concentration were detected among any of the three groups at any time (Table 1, Figure 1).

**Discussion**

Our study has demonstrated a differential effect of ischemic stroke on CSF neurochemistry in rats dependent on the cerebral hemisphere involved.

Human studies using acute poststroke measures of CSF biogenic amines and their metabolites have shown a generalized and lateralized increase in concentrations, with left-sided strokes being associated with greater increases. Whereas our present study has shown a lateralized effect of stroke, it is in a direction opposite to that seen in acute poststroke human studies. However, since our earliest measure in rats was 5 days after the stroke, these measures were not as close to the ischemic event as the 2–3 days after stroke in the human studies cited above. Other investigators who have studied changes in brain catecholamine concentrations at times much closer to the injury have reported that there is an initial increase in catecholamine concentrations within a few hours after surgery, followed by a more prolonged decrease.

Our findings of time-dependent depletion of monoamine metabolites following right MCA ligation is consistent with our earlier findings on the brain chemistry of rats with similar lesions. This supports the prevalent acceptance of CSF neurochemistry as a reflection of brain chemistry. In addition, the differences in CSF metabolites following right or left hemisphere ischemia are unlikely to have resulted from interhemispheric differences in lesion size or location since we have demonstrated that MCA ligation does not produce significant differences in mean lesion volume or location between the two hemispheres. It is also unlikely that differences in activity could account for these findings because the rats were restricted to a small cage for 24 hours before CSF sampling.

Although the relation between our findings in rats and those in humans is only speculative, it is perhaps relevant that in a recent study of chronic stroke patients we found a significant increase in $N$-methylspiperone binding as measured by positron emission tomography (PET) following right but not left hemisphere stroke.

In our present study, we found a significant difference in the levels of CSF 5-HIAA between rats with right and left hemisphere lesions 5 and 20 days after the stroke. These differences may have been due to a relative increase in the CSF 5-HIAA concentration in the left MCA ligation rats or a relative decrease in the right MCA ligation group or possibly to a combination of both these factors. Nonetheless, our findings of an asymmetric serotonergic response to cerebral ischemia in humans as well as in rats suggests that there may be important parallels between rat and human brains in their physiologic or neurochemical responses to stroke.

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