Direct Evidence of Acute, Massive Striatal Dopamine Release in Gerbils with Unilateral Strokes

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Dopamine release into the extracellular space was measured with in vivo electrochemical detection in the ipsilateral and contralateral striata in Mongolian gerbils that suffered a stroke after acute unilateral carotid artery ligations. A sevenfold increase in the dopamine signal occurred within 15 minutes of carotid ligation in the ischemic side, while the unlesioned side had no significant change. Increased extracellular levels of dopamine persisted throughout the 3-hour recording period. Pretreatment with α-methyl-p-tyrosine 6 hours prior to recording significantly attenuated the signal increase. This study is the first direct demonstration of the marked, continuous dopamine release that occurs during acute cerebral ischemia. (Stroke 1987;18:108-110)

THE neurotransmitter dopamine may be implicated in the tissue damage that occurs in the corpus striatum following cerebral ischemia. Dopamine is contained in high concentrations in the striatum, where it is found in nerve terminals originating from cell bodies in the substantia nigra. Pretreatment with α-methyl-p-tyrosine (AMPT) decreases damage to serotonin and glutamate nerve terminals, as well as to dopamine nerve terminals, suggesting that extracellular dopamine is toxic to the neuropil. Dopamine nerve terminals are selectively sensitive to ischemic damage.1,2 Dopamine levels in striatal tissue fall to 25% of control levels after 1 hour of ischemia,4-7 and similar losses have been demonstrated with histofluorescence techniques.8 The fate of the missing dopamine is unknown. Although this dopamine may have been released extraneuronally and subsequently metabolized, it is also possible that the release and metabolism occur intraneuronally through monoamine oxidase (MAO) or even by autoxidation.

Carotid occlusion in Mongolian gerbils has been employed extensively as a model of cerebral ischemia analogous to some forms of human stroke. Gerbils frequently have an incomplete circle of Willis, and unilateral ligation of the carotid artery produces cerebral ischemia in the ipsilateral half of the brain in 40% of the animals.9,10 To determine if ischemia induces extraneuronal dopamine release, we used in vivo electrochemical detection (IVED) to record changes in the level of extracellular dopamine in gerbils with acute cerebral ischemia. This technique, which has been reviewed elsewhere,11,12 measures brain concentrations of dopamine, norepinephrine, serotonin, and their metabolites, as well as of ascorbic acid and uric acid. The dimensions of the electrode are such that these measurements occur in the extracellular space and reflect concentrations of released substances.

Materials and Methods

The techniques used for IVED were similar to those described in detail previously.12 The working electrodes in this study were constructed from Teflon-coated silver wire (0.2 mm diameter) packed with carbon paste (1.0 ml silicon oil, 1.5 g Ultra F carbon powder). The reference electrode was a chloridized silver wire and the control electrode a silver wire attached to a cortical screw. One working electrode was stereotactically implanted in each corpus striatum (0.8 mm anterior to, ±2.8 mm lateral to, and 3.8 mm below the dura) of male Mongolian gerbils weighing 50-70 g. All electrochemical recordings employed voltammetry with semidifferentiation of the signal (Bioanalytical Systems DCV-5 Voltammetry Controller). Each striatum was scanned from -300 to +600 mV at a rate of 10 mV/sec at intervals of 15 minutes. The height above baseline of the major peak occurring at approximately 150 mV was measured.12

Following at least two days of recovery, each gerbil was connected to the IVED equipment and the recorded signal allowed to stabilize (about 3 scans). Some experiments were performed 6 hours after the intraperitoneal (i.p.) injection of 400 mg/kg AMPT. The gerbils were anesthetized with approximately 40 mg of methohexital/kg (i.p.), and the left common carotid artery was ligated.1 Each recording was continued for at least 3 hours with concurrent behavioral observation. Animals without evidence of definite strokes (evidenced by hemiparesis or rotational movement) were excluded from the study. Five gerbils with completed strokes were studied in both the untreated and AMPT-treated experiments. The unlesioned striatum served as a control in each animal, as did the measurements prior to carotid ligation. Electrode position was histologically verified at the completion of each experiment, as was the presence of stroke. The

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unpaired Student's t test was used to compare the AMPT-treated and untreated animals and the paired Student's t test to compare the lesioned and unlesioned side in the same animals.

**Results**

Figure 1 demonstrates typical changes in the voltammogram of a gerbil with stroke. As demonstrated in Figure 2, an average sixfold increase in peak height was recorded on the first scan from the side ipsilateral to carotid ligation (5.9 nA/sec$^{15}$ ± 1.0 (mean ± SEM) before vs. 31.9 nA/sec$^{15}$ ± 2.6 after ligation). The signal declined slightly but remained elevated over the next hour and then slowly increased over the next 2 hours. On the contralateral side the signal did not change significantly and tended to decrease slightly over the recording period.

When the gerbils were treated with AMPT (Figure 3), there was a slight but not significant decrease in the signal after 6 hours (4.4 nA/sec$^{15}$ ± 1.0 vs. 3.3 nA/sec$^{15}$ ± 0.4). The signal increased significantly after carotid ligation, but the rise was significantly less than that in untreated gerbils ($p<0.001$ for all the points). The signal in the AMPT-treated animals also increased from hour 1 to hour 3 of recording but was always significantly lower than the corresponding value in the untreated animals. The signal on the contralateral side did not change significantly, tended to decrease slightly, and did not differ statistically from the contralateral signal recorded in animals without AMPT.

**Discussion**

IVED directly measures extracellular (released) dopamine, whereas previous studies of the gerbil model measured whole-tissue dopamine, which includes a large intracellular component. The IVED technique also demonstrates the time course of dopamine release in the same animal because the animal does not have to be sacrificed to obtain the dopamine level at each time point. Each animal serves as its own control; experimental values are compared to the baseline (prestroke) signal and to the simultaneous recordings on the contralateral (nonischemic) side. The main disadvantage of IVED is that the signal recorded at 150 mV is, under some conditions, not entirely specific for dopamine.$^{11,12}$ However, the marked attenuation of the sig-

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Representative voltammogram from an untreated gerbil. Scan a was taken prior to ligation. Scans b, c, and d were taken 15, 45, and 120 minutes after ligation. The peak occurring at about 150 mV is marked with the arrow.

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Effect of unilateral carotid ligation of the dopamine signal. The zero time point is the average of the 3 signals measured before carotid ligation. Data points are averaged values from 5 animals. Error bars represent SEM. Currents have been semidifferentiated and are therefore in units of nA/sec.$^{15}$

![Figure 3](http://stroke.ahajournals.org/)

**Figure 3.** Effect of unilateral carotid ligation on the dopamine signal in AMPT-treated animals. The pre-AMPT data point is averaged from the 3 recordings preceding AMPT injection in all 10 gerbils. The zero time point was measured 6 hours after AMPT injection and is the average of the 3 signals measured before carotid ligation. Data points are averaged from 5 animals. Error bars represent SEM. Currents have been semidifferentiated and are therefore in units of nA/sec.$^{15}$
nal increase after treatment with AMPT (a tyrosine hydroxylase inhibitor) strongly suggests that, under the experimental conditions in the present study, the measurements do reflect dopamine release.

The study reported here is the first to directly demonstrate extracellular dopamine release. The initial release occurs within minutes of the stroke and may result from ischemic depolarization of membranes.6,7,13

The signal declines moderately before rising again, suggesting that some component of the dopamine store is rapidly released and that there may be a second component that is continuously released more slowly. Striatal dopamine appears to exist in two pools. AMPT inhibits tyrosine hydroxylase14 and reduces endogenous dopamine levels by preferentially eliminating the newly synthesized, readily releasable pool.15 A small storage pool of dopamine remains following administration of AMPT; the dopamine in this pool is depleted by reserpine and released by amfonelic acid.15,16 It is tempting to speculate that the marked signal attenuation of the initial peak in Figure 3 compared with Figure 2 is due to depletion of the AMPT-sensitive pool, while the continuing, slow rise of the signal in both Figures 2 and 3 may be due to release of the storage (AMPT-insensitive) pool. There does not appear to be any significant contribution to release of dopamine from anesthesia, surgical stress, or handling of the animals since the control signal did not change significantly throughout the study.

To our knowledge, this study is the first to demonstrate direct evidence of a marked extracellular dopamine release in the striatum of a conscious animal upon carotid ligation. It also demonstrates a striking attenuation of this release after catecholamine synthesis inhibition and is, therefore, an approach to monitoring pertinent neurochemical changes after pharmacological treatments.

References


Key Words • stroke • dopamine • in vivo electrochemical detection
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