Biphasic Striatal Dopamine Release During Transient Ischemia and Reperfusion in Gerbils

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To clarify the nature of ischemic striatal dopamine release during the earliest periods of neuronal injury, we used chronoamperometry to measure dopamine levels every 60 seconds during various durations of ischemia in 32 gerbils. Catecholamine-selective electrodes were implanted into the brains of anesthetized gerbils subjected to 2, 5, or 10 minutes of transient forebrain ischemia or permanent forebrain ischemia. Four control animals showed a stable chronoamperometric baseline. In the six gerbils subjected to permanent ischemia, dopamine release was rapid during early ischemia and slowed with time. The four animals subjected to 2 minutes of ischemia showed minimal dopamine release. The six gerbils subjected to 5 minutes of ischemia demonstrated a noticeable dopamine release during ischemia, and three of the six developed a massive secondary dopamine release during reperfusion. All six animals subjected to 10 minutes of ischemia demonstrated a similar biphasic dopamine release twice the size of that observed in the 5-minute group. Pretreatment with pargyline in six additional gerbils subjected to 10 minutes of ischemia demonstrated a similar biphasic dopamine release twice the size of that observed in the 5-minute group. Pretreatment with pargyline in six additional gerbils subjected to 10 minutes of ischemia failed to modify significantly this biphasic pattern of dopamine release. We conclude that dopamine release occurs very early during ischemia and that its magnitude correlates with the duration of an ischemic insult. Reperfusion is associated with an even larger striatal dopamine release. This previously unreported biphasic dopamine release phenomenon may have important clinical implications in the management of cerebral ischemia. (Stroke 1991;22:674–679)

Recently, attention has been focused on the role of neurotransmitters in the pathogenesis of ischemic cerebral injury. This area of research arose because investigators using microdialysis and in vivo electrochemical techniques in the striatum of experimental animals have long noted that a massive increase in the extracellular concentration of dopamine occurs shortly after death. Phebus et al 1 demonstrated striatal dopamine release shortly after respiratory arrest in rats. Brannan et al 2 used in vivo electrochemistry to demonstrate a massive unilateral striatal dopamine release in gerbils surviving unilateral stroke. Later, Slivka et al. 3 using the technique of microdialysis, confirmed the findings of Brannan et al. 2 Subsequently, Phebus and Clemens, 4 Globus et al, 5 and Ogura et al 6 demonstrated that ischemic periods as brief as 20–30 minutes effectively cause striatal dopamine release during the period of transient ischemia. Additionally, Globus et al 7 demonstrated that ischemic catecholamine release is not confined to the striatum, but is also seen in the hippocampus during transient ischemia.

A role for the ischemic release of catecholamines in neuronal injury was suggested by Clemens and Phebus, 8 along with Globus et al, 9 when they demonstrated that depletion of striatal dopamine prior to ischemia has a protective effect on the small and medium-sized neurons of the striatum. This conclusion was supported by the observations of Stein and Cracco 10 that the topical application of catecholamines to cortical tissue has toxic effects even in the
absence of ischemia. These investigations led Clemens and Phelbus and Globus et al. to propose that extracellular accumulation of dopamine, or its metabolites, may have a mechanistic role in the pathogenesis of ischemic striatal neuronal injury.

Significant ischemic damage has been demonstrated in the hippocampus with transient ischemic insults as brief as 5 minutes. If catecholamines do play a mechanistic role in the pathogenesis of ischemic damage, then it seems reasonable to expect that ischemic striatal dopamine release may begin to occur with transient ischemic insults as brief as 5 minutes. Previous studies have not concentrated on the nature of the earliest ischemia and reperfusion-related changes in striatal dopamine release. Understanding these initial neurotransmitter changes may help us understand the mechanisms of ischemic damage since these early changes undoubtedly reflect the primary cellular events that eventually lead to ischemic injury.

Therefore, we investigated the earliest ischemic striatal dopamine changes seen in gerbils undergoing bilateral carotid artery occlusion by using the in vivo electrochemical technique of chronoamperometry, which is capable of very short sampling intervals. Furthermore, we used only stearate-modified graphite paste working electrodes because of their well-documented preferential selectivity to catecholamines. These electrodes have been shown to accurately record changes in striatal dopamine levels without interference from ascorbic acid, uric acid, 3,4-dihydroxyphenylacetic acid (DOPAC), or homovanillic acid during many pharmacological manipulations.

Materials and Methods

Female Mongolian gerbils (Tumblebrook Farms, West Brookfield, Mass.) weighing 50–70 g were anesthetized with 400 mg/kg i.p. chloral hydrate initially, and the initial dose was supplemented as needed to maintain a surgical level of anesthesia. A midline neck incision was made, and both common carotid arteries were mobilized free from the surrounding tissue using microsurgical techniques. A strand of 5-0 silk suture was passed behind each artery, and the free ends of the sutures were then passed through a small flexible tube. Each suture thus formed a snare around each carotid artery that could be pulled and released at the appropriate times. The snares were adjusted to allow handling of the animals without restricting carotid artery blood flow. The position of the suture around the artery was carefully inspected after each manipulation of the snare.

The gerbils were placed carefully in a stereotactic apparatus. The skull was exposed through a midline scalp incision, and a small hole was drilled through the skull above the area of each striatum. The dura was carefully incised, and a stearate-modified graphite paste electrode was positioned in the right and left striatum (0.8 mm anterior and 2.8 mm lateral to the bregma, 3.6 mm below the dura, with the incisor bar set at −5 mm). A combined Ag/AgCl reference and stainless steel auxiliary electrode was placed in fluid contact with the brain using normal saline. The core temperature of each animal was monitored and kept at 37°C by a self-regulating heating pad.

The gerbils were connected to an in-house-built electrochemical device that was capable of linear potential sweep voltammetry using semidifferential electroanalysis and repetitive chronoamperometry. Semiderivative voltammograms (SDVs) were obtained by scanning from −200 to 350 mV at a scan rate of 10 mV/sec and a sampling time of 10 minutes. All animals were monitored initially following electrode placement using the SDV sampling mode until a stable SDV baseline was achieved (approximately 90 minutes).

Chronoamperometry was then performed using a 60-second-interval sampling time by applying a 1.0-second pulse from −100 to 250 mV (versus Ag/AgCl) to the working electrode. The oxidation currents were measured at the end of the 1.0-second pulse. The chronoamperometric baseline was recorded for approximately 15–20 minutes before the carotid arteries were occluded by pulling the free ends of the 5-0 silk sutures just enough to stop blood flow in both arteries. The occlusion was held in place for the required time and then released. A small mirror was placed near the front of the stereotactic apparatus so that visual verification of the occlusion and reperfusion could be made.

Thirty-two gerbils were divided into six groups. Four animals served as controls and underwent sham operations without carotid artery occlusion. Six underwent permanent forebrain ischemia induced by bilateral carotid artery ligation. Sixteen were divided into three groups and subjected to either 2, 5, or 10 minutes of transient forebrain ischemia induced by temporary bilateral carotid artery occlusion (n = 4, n = 6, and n = 6, respectively). An additional six gerbils were treated with 100 mg/kg s.c. pargyline 2 hours prior to 10 minutes of bilateral carotid artery occlusion.

The response to transient ischemia was recorded from the caudate nucleus of each animal until the chronoamperometric signal returned to baseline levels. In the permanent ischemia group, the signal was monitored for 40–180 minutes following carotid artery occlusion. The control group was monitored for 90 minutes following stabilization of the chronoamperometric signal. At the end of the study time, each animal was given an overdose of pentobarbital. Electrode placement was verified to be in the caudate by frozen section histology, using a standard cresyl violet stain.

Results

The four control gerbils showed a stable chronoamperometric signal over the 90 minutes of observation. This signal was essentially a flat line, despite needed supplemental anesthesia injections.
Figure 1. Top: Chronoamperometric recording showing effect of permanent bilateral carotid artery occlusion on caudate dopamine release in gerbils. Pattern is representative of the six animals in this group. Measurements were taken every 60 seconds. Bottom: Chronoamperometric recording showing effect of 2 minutes of temporary bilateral carotid artery occlusion and subsequent reperfusion on caudate dopamine release. Pattern is representative of the four animals in this group.

Figure 1, top, demonstrates a typical chronoamperometric response from one gerbil in the permanent ischemia group. This pattern was faithfully reproduced in all six animals. The signal began to rise around the third minute following occlusion. It then increased rapidly for the next 8±2 minutes (mean±SD), peaking at 11.3±1.0 nA above baseline. The signal fell for the next 5±2 minutes to 9.9±2.2 nA above baseline and then it began to rise again, but at a slower rate, eventually reaching 16.0±3.9 nA above baseline at 40 minutes of ischemia, the end of our study time. Monitored for 180 minutes in one gerbil, the level continued to rise at its present rate over that period. A similar result has been reported previously with electrochemistry.²

Figure 1, bottom, demonstrates a typical response from one animal subjected to temporary ischemia of 2 minutes' duration. A similar response occurred in all four gerbils of this group. The signal again tended to increase around the third minute following occlusion, which was the first minute of reperfusion. The response in this group was comparatively minimal and somewhat variable in magnitude and duration. The response reached only 1.1±0.2 nA above baseline at about 12±6 minutes after the start of the occlusion and then returned to baseline fairly quickly.

Figures 2, top, and 2, bottom, show the two different responses to 5 minutes of ischemia. Three of the six gerbils demonstrated a pattern similar to that shown in Figure 2, top, while the other three demonstrated a pattern similar to that shown in Figure 2, bottom. In the first pattern, reperfusion at 5 minutes was followed by an almost immediate plateau of the increasing signal at 6.0±2.0 nA above baseline 6±2 minutes after the start of the occlusion. The signal then slowly drifted upward for the next several minutes before taking a sudden drop toward baseline, falling to 1.9±0.8 nA above baseline 30±7 minutes after the start of the occlusion. The signal then climbed toward its previous value before peaking and gradually returning to baseline. In the other pattern, reperfusion did not suddenly stop the increasing signal as in the first three animals; rather, the signal continued to rise for the next few minutes peaking at 7.8±1.9 nA above baseline 8±1 minutes after the start of the occlusion. It then fell dramatically toward baseline, stopping short at only 1.6±1.5 nA above baseline 15±2 minutes after the start of the occlusion. The signal then displayed a second massive increase, which peaked at 15.3±1.7 nA above baseline 27±4 minutes after the start of the occlusion. The signal then gradually returned to baseline and remained there.
Examining the earliest ischemia-induced changes in extracellular striatal dopamine levels with the short-sampling-interval technique of chronoamperometry has revealed a number of new findings about the early ischemic dopamine release. First, dopamine release during permanent ischemia (i.e., stroke) occurs in a complex manner. This release shows a lag period before it starts, an initial rapid increase phase, an inflection point, and finally, a secondary slow increase phase. Second, transient ischemia of 2 minutes' duration is not a potent stimulus for dopamine release. Third, transient ischemia of 5 minutes' duration does cause the release of significant amounts of dopamine. Fourth, reperfusion following 10 minutes of transient ischemia is consistently associated with a massive secondary increase in dopamine release.

The mechanism by which ischemia causes increased levels of catecholamines in the extracellular space probably reflects both an increased release of dopamine into the extracellular space as well as a decreased removal of dopamine from the extracellular space. The synaptic release of catecholamines can be driven by increased extracellular potassium levels. Extracellular potassium levels are known to increase rapidly during ischemia, reaching levels of up to 60 mM within 3-4 minutes. This time frame overlaps the lag time seen in our present study and strongly implicates an early potassium-mediated release process. Removal of catecholamines from the extracellular space is largely due to neuronal reuptake, which is an adenosine triphosphate (ATP)-dependent process. Ischemia is known to cause ATP depletion within 5 minutes; therefore, within 5 minutes, dopamine reuptake from the extracellular space is blocked and the dopamine level in the extracellular space should increase. Furthermore, normal synaptic release is dependent on intracellular calcium levels. Ischemia is known to cause a rapid calcium influx, which in turn might be expected to drive synaptic catecholamine release by mass action alone.

The early rapid increase phase of dopamine release seen in the permanent ischemia group (Figure 1) is probably secondary to these energy-dependent changes. The fact that the early release shows a lag time and tends to start around the third minute of ischemia, rather than immediately, supports the argument that the early ischemic dopamine release is an energy-dependent phenomenon. This lag period suggests that time is required before energy depletion reaches some threshold value and reuptake begins to slow down.

The inflection point occurring around 8 minutes (Figure 1) may represent a maximum in the release from the pool of newly synthesized, readily releasable dopamine. The magnitude of the release at the inflection point is 70.6% of that at 40 minutes. This suggests that the majority of ischemic dopamine release occurs during the first 8 minutes of an ischemic insult. This point may prove to have important clinical implications (i.e., determining the proper timing of drug delivery during cardiopulmonary resuscitation, carotid endarterectomy, and acute stroke).

After the inflection point (Figure 1), the dopamine level again rises, but at a much slower rate. The slow rise seen with this late release phase may simply represent a continuation of the energy-dependent changes as the last bits of dopamine are released, or it may signify a change in the type of release occurring. It is possible that the large influx of
Calcium activates membrane phospholipases by this time. These phospholipases, known to directly injure cell membranes during ischemia, may also damage the intracellular dopamine storage vesicles, causing the remaining stored dopamine to slowly leak out of the cell.

The dopamine release seen during 2 minutes of bilateral carotid artery occlusion was minimal compared with the release seen in the other groups (note scale markers in all figures). However, the magnitude of the change is comparable to those usually recorded with stearate-modified electrodes during normal pharmacological manipulations of the striatal dopamine system in rats. Such manipulations rarely cause a current change exceeding 1 nA in magnitude. The magnitude of the dopamine release seen in the 2-minute ischemia group is, therefore, not considered to be of a pathological size. This observation is consistent with the "dopamine" hypothesis of ischemic damage because 2 minutes of ischemia is known not to cause significant neuronal damage. If, on the other hand, a massive dopamine release was seen with this very brief stimulus, it would strongly suggest that dopamine release is caused by nonspecific factors.

The magnitude of the release in the 5-minute ischemia group, measured at the first peak, is about five to seven times that in the 2-minute ischemia group. The magnitude of the release is clearly beyond that caused by normal pharmacological manipulations of the striatal dopamine system. The two separate and distinct patterns of dopamine release seen with 5 minutes of ischemia are probably due to variability in the gerbil cerebral circulation. Approximately 30–40% of gerbils lack a significant anterior communicating cerebral artery and are prone to stroke following unilateral carotid artery ligation. Bilateral carotid artery occlusion consistently produces bilateral forebrain ischemia in all animals since gerbils lack a posterior communicating cerebral artery connecting the vertebrobasilar and carotid artery systems. And, we know that ischemia can occur with carotid artery occlusions as brief as 5 minutes.

Our data suggest that the gerbils with the lesser dopamine release (Figure 2, top) have some, although limited, collaterals between the posterior and anterior cerebral circulations and thus suffered a milder ischemic insult during the brief ischemic period of 5 but not 10 minutes.

Our data also suggest that researchers using the gerbil model should adopt a standard duration of transient ischemia to allow future studies to be directly comparable. We suggest that 10 minutes of transient ischemia would be preferable to 5 minutes for bilateral carotid artery occlusion because of the variability seen in the 5-minute group and the uniform response seen in the 10-minute group.

The magnitude of the dopamine release in the 10-minute group, measured at the first peak, is about 11 times that in the 2-minute group and almost double that in the 5-minute group. The pattern of release suggests that the degree of ischemic damage is proportional to the magnitude of dopamine release. However, histological studies are necessary to confirm the correlation.

Previous investigations using microdialysis in the rat hypotensive model of transient ischemia or the rat four-vessel occlusion model have confirmed that the first chronoamperometric peak seen during ischemia is due to dopamine. However, no obvious reperfusion effects on striatal dopamine have been reported. In fact, Damsma et al reported that reperfusion was associated with a massive increase in the concentration of DOPAC, and this DOPAC release demonstrated a time course and magnitude paralleling the shape of the second chronoamperometric peak seen in our present study.

However, in our present study, pretreatment with pargyline (which blocks the conversion of dopamine to DOPAC) failed to eradiate the second dopamine release during reperfusion, suggesting that the biphasic response is indeed predominantly due to dopamine. The failure of previous investigators to detect this biphasicity may be due to at least two factors. First, the microdialysis techniques require a sampling time of 20 minutes, and the rise and fall of dopamine concentration could have been lost in the long sampling intervals. Second, these previous studies used a completely different animal model, the rat, the abundant cerebral collateral circulation of which often requires four-vessel occlusion and induced hypotension to produce significant cerebral ischemia. In the previous microdialysis studies, ischemia was induced using four-vessel occlusion alone. Obrenovitch et al recently showed that 10 minutes of ischemia induced by four-vessel occlusion without hypotension elicited a large catecholamine rise in only six of 11 rats; the other five rats showed a minimal response similar to that of our gerbils subjected to only 2 minutes of ischemia. Furthermore, it is interesting that Obrenovitch et al observed that one rat subjected to 20 minutes of ischemia revealed "an interesting biphasic change in its striatal catecholamine level during ischemia, ... an initial transient rise, ... followed by a massive increase"—a pattern very similar to the one we report.

Additionally, the biphasic nature of the release looks very much like the biphasic pattern of release seen following the administration of 10 mM 1-methyl-4-phenylpyridinium (MPP+) intrastriatally in rats. MPP+ is known to be the cytotoxic metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a drug that causes a parkinsonian-like syndrome in primates. Why MPP+ causes a biphasic striatal dopamine release is not known, but comparison with our present data suggests that a toxic metabolite may accumulate during ischemia and produce its effect during subsequent reperfusion.

Finally, this study suggests that chronoamperometry may play a useful role in the investigation of different pharmacological agents used for ischemic protection. For instance, one could test the hypoth-
esis that a calcium channel blocker, such as nimodipine, changes the slope or magnitude of the early dopamine release and perhaps even attenuates the reperfusion response. In fact, Uematsu et al. have already shown that nimodipine attenuates both increase in cytosolic free calcium and histological damage following focal cerebral ischemia and reperfusion in cats. Further studies to understand and modify this reperfusion phenomenon are warranted.

In conclusion, we have demonstrated that a relation exists between the duration of an ischemic insult and the magnitude of striatal dopamine release. This relation is consistent with the proposed mechanistic role for extracellular dopamine in the pathogenesis of ischemic injury. Two minutes of ischemia had little effect on dopamine release, whereas 10 minutes of ischemia consistently caused a massive biphasic release, with the larger peak occurring during reperfusion. These data suggest that critical events occur between 2 and 10 minutes of ischemia and during reperfusion. Investigating the nature of this critical period is greatly facilitated by the in vivo electrochemical technique. Future studies using this technique are needed to further elucidate the mechanism of ischemic neuronal injury and to understand the biphasic reperfusion catecholamine response.

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