Changes in Striatal Dopamine Metabolism Measured by In Vivo Voltammetry During Transient Brain Ischemia in Rats

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In vivo voltammetry was used in rats with brain ischemia induced by four-vessel occlusion to measure changes in dopamine metabolism via measurement of peak 2 (dopamine compounds) in the striatum. Changes in regional cerebral blood flow in the striatum were also assessed by means of a temperature-controlled thermoelectrical device. Peak 2 increased by 600-900% during 30 minutes of four-vessel occlusion, which may have reflected an ischemia-provoked increase in the release of dopamine and a disturbance in the outward transport of its metabolites. Following reperfusion by discontinuation of carotid occlusion, peak 2 rapidly decreased to below control values and then gradually increased, exceeding control values at 180-210 minutes after reperfusion. Regional cerebral blood flow in the striatum decreased to almost 0 ml/100 g/min during the ischemic period, transiently increased to greater than control values after reperfusion, then gradually decreased during the next 240 minutes. Since dopamine is known to have various effects on cerebral metabolism and blood flow, alterations in its behavior may contribute to changes in cerebral blood flow and to postischemic brain damage. In vivo voltammetry may be useful in the investigation of the pathophysiology of brain ischemia. (Stroke 1989;20:783-787)
Male Sprague-Dawley rats weighing 250–300 g were anesthetized with 50 mg/kg pentobarbital before implantation of the electrodes. The working electrode was inserted into the striatum, according to the coordinates of Pellegrino et al.19 (anterior, 1.0 mm from the bregma; lateral, 3.0 mm from the midsagittal line; and vertical, 4.5 mm from the dura). Reference and auxiliary electrodes, one each, were placed on the dural surface of the frontal region and, along with the plug connected to them, were affixed to the skull with dental cement.

The rats were divided into nonischemic (control) and ischemic groups. In the latter, according to the four-vessel occlusion model of Pulsinelli et al.20–22 the vertebral arteries were electrocauterized through the alar foramina of the first vertebra at the time of electrode implantation. In both groups, recording was stated approximately 48 hours after electrode implantation because oxidation peaks obtained in the brain were unstable just after implantation. We measured peak 2. Peaks 1 (ascorbic acid) and 2 were discriminated by the method of Ikeda et al.15 Specifically, peak 1 was eliminated by holding the potential (50 mV) between the reference and working electrodes at an oxidation potential higher than that of ascorbic acid (−50 mV) for 1 minute before each measurement.

In the control group, recordings were obtained at 15-minute intervals for 4 hours in nonischemic rats with (n=5) and without (n=5) pentobarbital anesthesia.

In the ischemic group (n=5), the common carotid arteries were exposed and temporarily occluded by clipping, and the rats were thus subjected to 30 minutes of brain ischemia. IVV recordings were obtained every 10 minutes. Reperfusion was accomplished by release of the carotid clamps, and IVV recordings were made every 10–30 minutes from 10 to 210 minutes after reperfusion. During this procedure, one femoral artery was cannulated for measurement of mean arterial blood pressure (MABP) and blood gas analysis. At the end of each experiment, the locations of the electrode tips were verified histologically.

The results are expressed as percentages of the mean control value, which was calculated by averaging the five initial preischemic absolute values of peak 2 in anesthetized rats. Statistical analysis was made by Student’s t test. All values are expressed as mean±SEM.

In addition, changes in CBF were assessed by a temperature-controlled thermoelectric device (UMW-101, Unique Medical Co., Ltd., Tokyo, Japan) placed in the striatum. This technique, which has been reviewed elsewhere,23 consists of two gold plates the temperature difference of which is maintained at 2°C by a self-regulating system. The power required by the microheater to maintain this temperature difference reflects CBF.

Results

Voltammograms recorded in vitro from PBS at pH 7.3 containing DA or DOPAC showed distinct oxidation peaks for DA and DOPAC, both of which appeared at 150 mV of the oxidation potential (Figure 1).

Voltammograms obtained from the striatum of freely moving rats yielded oxidation peaks 2 and 3 (peak 1 was eliminated as described above). Peak 2 appeared at the same potential as DA and DOPAC (Figure 1). Peak 3 was not measured. Peak 2 remained well defined and very stable during the 4 hours of recording in control rats, and pentobarbital anesthesia induced no significant changes in the peak 2 height (Figure 2).

Although preparation of the rats by the method of Pulsinelli and Brierley calls for a 24-hour delay after cauterity of the vertebral arteries, we induced forebrain ischemia 48 hours later to allow stabilization of the implanted electrodes. The changes in CBF and MABP are shown in Figure 3; regional CBF in the striatum decreased to almost 0 ml/100 g/min.
during four-vessel occlusion. The striatal peak 2 markedly increased during forebrain ischemia (Figures 4 and 5). The increase was significant (p<0.05, 600–900%) during the early stage of ischemia (within 10 minutes) and decreased gradually over the following 20 minutes.

When reperfusion was initiated by removing the clips from the common carotid arteries, peak 2 decreased to 70–80% of the control value within approximately 60–90 minutes (p<0.01; Figure 5). From that time, peak 2 gradually increased, exceeding the control value after 180 minutes (difference not significant) and becoming significantly higher at 210 minutes (p<0.05). CBF transiently increased immediately after reperfusion, then gradually decreased (Figure 3); it remained below the preischemic level until 240 minutes after reperfusion.

Blood gas analysis revealed that pH, PCO₂, and PO₂ were within physiologic limits, with no significant changes from the preischemic values.

Discussion

The four-vessel occlusion model developed by Pulsinelli et al.20-22 is technically simple and produces virtually complete ischemia in the striatum. Although there has been debate about the variability of ischemia in this model,24 our CBF data confirmed the presence of severe ischemia despite our having modified the model.

When cerebral ischemia occurs, the alterations in intracellular energy enhance nonphysiologic release of neurotransmitters in association with an increase in the intracellular calcium concentration. Mršulja et al.4 demonstrated an increase in the release of [³H]norepinephrine in synaptosomal preparations of ischemic brain. Since tyrosine hydroxylase (TH), dopamine-β-hydroxylase, and monoamine oxidase (MAO) require molecular oxygen for their activities, DA synthesis and degradation are believed to decline during severe hypoxia, a theory that has been supported by a number of experimental studies.1,6,7,10 However, the activity of TH may accelerate during mild hypoxia.5,8
On the other hand, in cerebral ischemia MAO activity decreases, resulting in a disturbance of monoamine metabolism. Consequently, the reuptake of released monoamines, which depends on energy metabolism (i.e., ATP), decreases. Mršulja et al speculated that the transport and excretion of metabolites of neurotransmitters are impaired in brain ischemia since the changes in homovanillic acid and 5-hydroxyindoleacetic acid (5-HIAA) resemble those induced by the administration of probenecid.

In most previous studies, both intracellular and extracellular monoamines were found throughout the brain after death following ischemia or reperfusion. This finding is thought to reflect changes in the synthesis, release, transport, and degradation of these neurotransmitters. Using IVV, we determined serial changes in DA metabolism in the extracellular spaces of the striatum of live rats during forebrain ischemia and reperfusion.

Several studies have revealed that peaks 1, 2, and 3 in IVV recording from rat striatum represent ascorbic acid, DOPAC, and 5-HIAA, respectively. However, peak 3 may contain uric acid as well, and in vitro DA may also be represented by peak 2 at the same potential. The steady-state extracellular DA concentration is too low to be easily detected by voltammetry, and previous work has disclosed that changes in peak 2 correspond to those of DOPAC. Therefore, DOPAC may well be the major contributor to peak 2. During abnormal conditions, such as cerebral ischemia, however, the origin of peak 2 is obscure. Phebus et al, using IVV, observed a large increase in electrochemically reactive materials in rats following their death and identified the reactive material corresponding to DA using in vivo brain dialysis. Brannan et al reported that the DA signal increased after brain ischemia in rats is attenuated by pretreatment with α-methyl-p-tyrosine.

In our study, peak 2 increased sixfold-to-ninefold during the early phase of ischemia and remained elevated during the entire 30-minute ischemic period. This finding is consistent with the reports concerning DA release during anoxia and ischemia as measured by IVV and suggests that DA accumulates in the extracellular spaces of the striatum as a result of its markedly accelerated release and impaired outward transport and reuptake due to brain ischemia. During brain ischemia, the elevation of peak 2 corresponds to changes in DA concentration. However, alterations in peak 2 after reperfusion may not reflect concentrations of DA alone. It is possible that peak 2 originates from compounds containing both DA and DOPAC. The rapid decrease in peak 2 height after reperfusion, to lower than the control level within 60–90 minutes, may indicate that the recovery of CBF-induced rapid transport of DA compounds that had accumulated in the extracellular spaces and that DA synthesis, release, and degradation were still impaired during the early phase of reperfusion.

Peak 2 began to increase gradually 60 minutes after reperfusion and exceeded the control value at 180–210 minutes. The second increase may have been due to irreversible, ischemia-induced disruption of the synaptic membrane, a decrease in receptor concentration, and a reduction in reuptake at the synapse, as Jellinger and Riederer have speculated. It is also possible that when CBF recovers, the synthesis, release, and degradation of DA are accelerated over time, leading to its accumulation in the extracellular spaces. Calderini et al found that the intracerebral tyrosine and DA concentrations did not decrease during ischemia, but increased.
significantly during the ensuing 30-minute reperfusion, suggesting that DA synthesis increased following reperfusion.

It is likely that changes in the synthesis, release, degradation, and reuptake of monoamine neurotransmitters caused by ischemia and reperfusion affect CBF and cerebral metabolism, including clinical manifestations. In their model, Pulsinelli et al.22 observed that glucose metabolism in the striatum remained low from 30 minutes after the induction of ischemia to 48 hours after reperfusion. In contrast, striatal CBF temporarily increased for up to 15 minutes after reperfusion, following a marked decrease during the ischemic period, and remained lower than the control value from 30 minutes to 6 hours after reperfusion. These CBF changes were similar to those we observed for up to 4 hours after reperfusion, using the controlled thermoelectrical method. The pattern of fluctuation, as determined by IVV, resembled the peak 2 changes, although the time courses differed slightly. Thus, there may be a causal relation between CBF changes and DA metabolism. The transient increase in CBF following reperfusion may be a consequence of the accumulation of DA or DOPAC during ischemia, and these monoamines may also be involved in the CBF changes that occur after 30 minutes of reperfusion.

Our results demonstrate that IVV permits detection of changes in DA metabolism in microregions of ischemic brain and their relation to cerebral metabolism and CBF. IVV can also be used to determine the effects of various drugs on cerebral ischemia.

References


KEY WORDS: cerebral blood flow • cerebral ischemia • dopamine • rats
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Stroke. 1989;20:783-787
doi: 10.1161/01.STR.20.6.783

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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