Focal Ischemia Enhances Choline Output and Decreases Acetylcholine Output From Rat Cerebral Cortex

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Choline concentration is rate limiting in the synthesis of acetylcholine. There is a negative arteriovenous difference for choline concentration across the brain, indicating the steady output of choline from this organ. Cerebral ischemia may increase extracellular choline concentration by interfering with its removal by the circulation and by enhancing its net production from phospholipids. We tested this hypothesis in six rats subjected to middle cerebral artery occlusion. We determined choline and acetylcholine output from the ischemic cerebral cortex by analyzing their concentrations in the fluid contained in cortical cups by gas chromatography-mass spectrometry. Mean±SEM choline output over 40 minutes before ischemia (baseline value) was 31.1±1.6 pmol/min/cm². During ischemia, mean±SEM choline output rose to 100.8±13, 97.3±12.7, 100±22.4, and 93.1±16.9 pmol/min/cm² in four consecutive 10-minute periods, respectively. Mean±SEM acetylcholine output was 15.6±1.1 before and 5.9±1.2, 8.3±2.6, 8.6±2.1, and 13.7±4.6 pmol/min/cm² in the four 10-minute collection periods during ischemia. All four choline values and the first acetylcholine value during ischemia were significantly different from their respective baseline values. We conclude that ischemia induces an increase in extracellular choline concentration with possible implications for acetylcholine metabolism. The attending transient decline in acetylcholine output may be due to impaired release due to local hypoxia or to decreased acetylcholine synthesis. (Stroke 1989;20:92-95)

Free choline (Ch) can be normally found in the brain extracellular and intracellular spaces. Ch concentration is rate limiting in the synthesis of acetylcholine (ACh).1-3 Previous data suggest that brain tissue sustains a steady output of Ch since a positive arteriovenous difference for this molecule has been found.4-7 Since it is known that ischemia triggers lipid degradation in the brain,8 it is conceivable that ischemia may increase extracellular Ch concentrations, both by enhanced production from phospholipid breakdown and by decreased removal by the circulation, although there is no experimental proof that this is the case; there is no information in the literature about the behavior of ACh release with cerebral ischemia, either. Since Ch availability is rate limiting for the synthesis of ACh, the increased extracellular concentration of this precursor, expected to occur in ischemia, would tend to increase the rate of synthesis and, secondarily, the release of ACh. On the other hand, ischemia may decrease the availability of acetyl-coenzyme A and by that mechanism decrease synthesis of ACh.

To gain insight into these potential effects, we subjected six rats to focal cortical ischemia by occlusion of the middle cerebral artery (MCA). Ch and ACh output into cups placed over the pial surface of the exposed cortex was determined by analyzing their concentration in the fluid contained in these cups after a suitable equilibration period. We have followed the essentials of the technique originally described by MacIntosh and Oborin.9 Ch and ACh output from the cerebral cortex was determined before and at various times after MCA occlusion.

Materials and Methods

Six adult male Sprague-Dawley rats (300–350 g body wt) were anesthetized with halothane (2.5% induction and 1.5% maintenance) vaporized in a mixture of 70% nitrous oxide and 30% oxygen. A femoral vein and artery were cannulated to allow fluid replacement, drug administration, and record-
Cerebral cortical blood flow (rCBF) was measured in nine rats under anesthesia and experimental conditions identical to those used for Ch and ACh output determinations. In five rats, the MCA was occluded 20 minutes before rCBF was determined, and the remaining four rats were used as controls (no MCA occlusion). The autoradiographic iodol[14C]antipyrine (IAP) technique was used as described by Sakurada et al. IAP (100 μCi/kg, specific activity 50 mCi/mmol, Amersham Corp., Arlington Heights, Illinois) was infused intravenously over 30 seconds. Timed samples of arterial blood were obtained every 2–3 seconds throughout the IAP infusion for the determination of radioactivity. An intravenous bolus of saturated KCl, administered through a second intravenous line during the last 2 seconds of IAP infusion was used, instead of decapitation, to terminate the experiment. This was necessary because the rat’s head was held in a stereotactic head holder that prevented the use of a guillotine. The exact timing of circulatory arrest was determined from a continuous record of arterial blood pressure. The brain was then rapidly removed, frozen in methylbutane chilled to −70°C and sectioned in 20-μm slices on an American Optical-Reichert Histostat cryostat (Buffalo, New York) at −20°C. Sections were heat-dried and exposed to Kodak AR-Xomat film (Rochester, New York) in x-ray cassettes for 1 week along with eight standards of known radioactivity. After developing, optical density of images induced on the film by brain sections and standards was determined with a microscope-based, computerized densitometer. The system was programmed to calculate tissue radioactivity and then tissue blood flow after data on the time course of blood IAP activity and final tissue activity were entered into the operational equation described by Kety.14

Results

 Autoradiograms showed an area of ischemia over the somatosensory cortex in the five rats subjected to MCA occlusion (Figure 1). Mean±SEM blood
flow within the area covered by the cortical cup was 0.47±0.06 ml/g/min, a decrease of 60% compared with the value of 1.20±0.19 ml/g/min measured in the nonischemic, symmetric area of the contralateral cortex.

Mean±SEM Ch baseline outputs during the four collection periods before MCA occlusion were 36±2.3, 32.7±1.5, 28.1±3.1, and 27.7±4.6 pmol/min/cm², respectively (Figure 2). MCA occlusion (conditions that decreased rCBF by 60%) induced an increase in cortical Ch output to 100.8±13 pmol/min/cm² during the first collection period after occlusion and to 97.3±12.7, 100±22.4, and 93.1±16.9 pmol/min/cm² after the second, third, and fourth periods, respectively.

ACh output was significantly decreased after MCA occlusion. Mean±SEM ACh baseline outputs during the four collection periods before occlusion were 17.9±2.7, 16.7±0.9, 15±2.2, and 12.7±2.5 pmol/min/cm². During the periods following MCA occlusion, mean±SEM outputs were 5.9±1.2, 8.3±2.6, 8.6±2.1, and 13.7±4.6 pmol/min/cm² (Figure 2).

Discussion

Distal occlusion of the MCA brought about a decrease of rCBF in its area of distribution to 0.47±0.06 ml/g/min. This is in line with previous observations from our laboratory and other laboratories.

This level of rCBF is above the threshold for infarct development in rodents, cats, and rabbits suggested that ischemia could induce a similar change since this condition is accompanied by increased PCO₂ in tissue. That was not the case, however, in our experiments. It is conceivable that the levels of hypoxia attained were well within the range that interferes with synaptosomal ACh release. Previous observations in which hypercapnia enhanced ACh output from the cerebral cortex in cats and rabbits suggested that ischemia could induce a similar change since this condition is accompanied by increased PCO₂ in tissue. That was not the case, however, in our experiments. It is conceivable that the enhanced ACh output induced by hypercapnia may be caused by activation of a subcortical cholinergic system with cortical projections rather than by a local cortical mechanism. Some support for this interpretation is provided by the experiments of Hudson et al., in which precollricular decerebration was shown to prevent the enhanced ACh cortical output associated with hypercapnia.

Which of the two basic mechanisms (decreased synthesis or impaired release) explains the decreased cortical output of ACh in our experiments cannot be ascertained with the available data and will require further investigation.
determination of ACh concentration in tissue. The relative contributions of decreased circulatory clearance and enhanced net production from phospholipids to the excess Ch observed in cortical ischemia will probably be clarified by measuring the amount of Ch removed from the brain by the circulation during normal and reduced perfusion.

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**References**


**Key Words** • acetylcholine • cerebral ischemia • choline • rats
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