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

Oscar U. Scremin, MD, PhD, and Donald J. Jenden, MD, PhD

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)
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 iodo\textsuperscript{14}C\textsuperscript{+}Clampyprine (IAP) technique was used as described by Sakurada et al.\textsuperscript{13} IAP (100 \textmu Ci/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-\textmu m 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.\textsuperscript{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 our14 and other laboratories.16-20 This level of rCBF is above the threshold for infarct development in rodents.21-24 We chose this type of MCA occlusion to produce ischemic changes of the type observed in the "penumbra," an area of reduced blood flow and compromised function but without permanent damage.15-24 MCA occlusion was associated with a marked increase in Ch output that may be explained by both enhanced production of unesterified Ch within the brain and decreased Ch removal by the circulation from brain extracellular fluid. As to the source of excess Ch, we can safely exclude ACh breakdown since physostigmine was added to the fluid superfusing the cortex. Increased breakdown or reduced synthesis of membrane phospholipids is probably the main factor to be considered. Incubation of isolated brains or brain homogenates at 37° C, an extreme model of ischemia, induces formation of large amounts of free Ch, but only in subcellular fractions of brain that contain membranes.25 Under such experimental conditions, phosphatidylcholine was the main source of free Ch. We can speculate that the same process may occur in our experiments. However, the relative contributions of Ch-containing phospholipid breakdown products and decreased removal by the circulation to the excess Ch output we observed cannot be ascertained with the available data.

A transient decrease in ACh output followed MCA occlusion; there are many possible mechanisms that may explain this phenomenon. First, interference with the release mechanism itself by the conditions that accompany cerebral ischemia, such as decreased pH,20-28 decreased availability of energy substrates, and decreased interstitial calcium activity,29 may be responsible. Second, it is possible that in spite of excess Ch, ACh synthesis may have diminished as a consequence of local hypoxia. Gibson and Blass30 have provided evidence of impaired ACh synthesis in systemic hypoxia. In vitro experiments have shown a threshold of 20 mm Hg PO₂, below which ACh release from synaptosomes is impaired. This is slightly higher than the threshold for lactate production (16 mm Hg).31 At the rCBF levels observed in our preparations, MCA occlusion is known to induce significant lactic acidosis.20-28 Therefore, it is likely that the levels of hypoxia attained were well within the range that interferes with synaptosomal ACh release.

Previous observations32,33 in which hypercapnia increased 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.34 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,35 in which precollicular 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
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.

Acknowledgments

The authors are grateful for the excellent technical assistance provided by Margareth Roch, Kathleen Rice, and Catherine Torres.

References

31. Park IR, Bachelard HS: Threshold requirements for oxygen in the release of acetylcholine from, and in the maintenance of the energy state in, rat brain synaptosomes. J Neurochem 1987;49:781–788

Key Words • acetylcholine • cerebral ischemia • choline • rats
Focal ischemia enhances choline output and decreases acetylcholine output from rat cerebral cortex.

O U Scremin and D J Jenden

*Stroke*. 1989;20:92-95
doi: 10.1161/01.STR.20.1.92

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/1/92

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/