Time-Dependent Changes in Cerebral Choline and Acetylcholine Induced by Transient Global Ischemia in Rats

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We occluded the carotid and vertebral arteries of 12 rats for 15 minutes to measure the brain concentrations of choline and acetylcholine and cerebral blood flow at the end of the ischemic period or 15, 30, or 150 minutes after circulation was reestablished. The animals were sacrificed with microwave radiation focused to the head immediately after a brief infusion of [14C]iodoantipyrine with rapid sampling of arterial blood. Brain tissue samples were extracted with ether to separate the tracer, which was subsequently measured by liquid scintillation counting and used to calculate local cerebral blood flow. The aqueous phase was then processed for the measurement of choline and acetylcholine concentrations by gas chromatography/mass spectrometry. The results showed a large increase in tissue choline content and a decrease in tissue acetylcholine content during ischemia. During recirculation, choline levels progressively declined, reaching levels lower than those in four control rats after 150 minutes of recirculation for most brain regions. A reciprocal relation between the brain choline concentration and local cerebral blood flow was found. Acetylcholine levels showed an initial rebound to greater than control during recirculation, with subsequent normalization. Brain acetylcholine concentration was positively correlated with brain choline concentration, provided that cerebral blood flow was >0.3 ml·x·g⁻¹·min⁻¹. Because tissue free choline was depleted in most brain regions 150 minutes after transient ischemia, we speculate that prolonged ischemia may produce a greater depletion of tissue free choline with a resulting decline in tissue acetylcholine. This could play an important role in the cognitive deficit associated with vascular dementia. (Stroke 1991;22:643-647)

Acetylcholine is a neurotransmitter of crucial importance in the central nervous system. Only a few studies have addressed alterations in the cerebral concentrations of this molecule under ischemic conditions. The concentrations of both precursors of acetylcholine, choline and acetyl-coenzyme A (acetyl-CoA), are known to be affected by the level of cerebral blood flow (CBF). Within the brain, choline is continuously produced by hydrolysis of phospholipids and acetylcholine and reutilized for synthesis of phospholipids and acetylcholine. These processes normally result in a net production of free choline that is washed away by the circulation and is reflected in a higher cerebral venous than arterial concentration. As a consequence, an inverse relation between CBF and tissue choline level occurs during focal cerebral ischemia. This effect tends to enhance the rate of acetylcholine synthesis by a precursor-loading mechanism when CBF decreases. Acetyl-CoA used for acetylcholine synthesis, on the other hand, originates from pyruvate, the concentration of which is known to decrease during ischemia. Thus, a reduction of CBF will potentially affect acetylcholine synthesis in opposite ways by changing the availability of its precursors.

Transient forebrain ischemia induced by occlusion of the vertebral and common carotid arteries in rats is a well-characterized model. Brain function is severely impaired during four-vessel occlusion, followed by restoration of function upon reestablishment of circulation if the ischemia is not prolonged beyond a critical period. We used this model to characterize the relations between local CBF and the choline and acetylcholine concentrations in brain tissue during transient cerebral ischemia and recirculation.

Materials and Methods

We used 16 male Wistar rats (Hilltop Laboratory Animals, Inc., Scottsdale, Pa.) weighing 250–300 g.
All surgical procedures were performed under halothane anesthesia (2.5% in air for induction and 1.5% in air for maintenance). The vertebral arteries were cauterized at the level of the alar foramina of the atlas and two snares, each made of a 2-0 silk thread encased in a segment of PE-160 polyethylene catheter (Intramedic), were passed around the common carotid arteries, which had been exposed by dissection of the neck. The snares were exteriorized through small openings in the back of the rat’s neck. Two femoral veins and arteries were cannulated and exteriorized through skin wounds that were sutured with 5-0 silk.

After all surgical procedures were completed, the rat was transferred to a nonstressing restraining device (Bollman cage) and allowed to recover from anesthesia for 90 minutes. Rectal temperature was continuously monitored and maintained between 36.5° and 37.5°C with the aid of a source of radiant heat. Global cerebral ischemia was induced by closing the snares around the carotid arteries. Temporarily freed from its Bollman cage, the rat’s ability to right itself from a supine position and maintain equilibrium on its four legs was tested. If this reaction was present, the rat was immediately euthanized with intravenous T-61 euthanasia solution [200 mg/ml n-2-(m-methoxyphenyl)-2-ethylbutyl-1-γ-hydroxybutyramide, 50 mg/ml 4,4-methylene-bis(cyclohexyltrimethyl)ammonium iodide, and 5 mg/ml tetracaine hydrochloride; American Hoechst Corp., Somerville, N.J.]. If the rat was not able to right itself but maintained spontaneous respiration, it was returned to its Bollman cage and the four-vessel occlusion was maintained for 15 minutes, after which time the snares were opened.

At the prescribed time after the end of the ischemic period, the Bollman cage containing the rat was introduced in the animal chamber of a 5-kW microwave fixation device (Biostat, Gerling Instruments, Modesto, Calif.). One arterial catheter was connected to a pressure transducer for the recording of arterial blood pressure (BP), one venous catheter to a syringe containing the blood flow tracer [14C]iodoantipyrine ([14C]IAP), and the other venous catheter to a syringe containing the euthanasia solution. The second arterial catheter was used for the sampling of arterial blood every 2–3 seconds during tracer infusion. The procedure was initiated by starting the infusion of [14C]IAP at a rate of 100 μCi×kg⁻¹×min⁻¹ and maintaining it during 0.5 minutes. During the last 3 seconds of infusion, the euthanasia solution was injected. This induced a precipitous drop of BP, and an observer watching the BP tracing activated the microwave power when BP reached 50% of its initial level.

The brain was then rapidly removed and frozen. Two brain slices were produced by coronal cuts at 1 mm behind and at 5 and 6 mm behind the bregma. Regions were dissected with a knife from both cerebral hemispheres in these two slices (weight range 10–20 mg) and homogenized in ice-cold 13% formic acid, 85% acetone containing deuterium-labeled internal standards for choline and acetylcholine assay. Acetylcholine, choline, and [14C]IAP were extracted from these homogenates and assayed by gas chromatography/mass spectrometry (acetylcholine and choline) or liquid scintillation counting ([14C]IAP) as described in detail elsewhere.3 Regions dissected were the cingulate cortex, medial frontoparietal cortex, lateral frontoparietal cortex, olfactory cortex, rostral hypothalamus, medial striatum, lateral striatum, hippocampal CA1 region, and hippocampal CA2 region. All regions were sampled in both cerebral hemispheres. For every region, two samples were obtained in each rat, with the exception of the lateral frontoparietal cortex, for which four samples per rat were obtained. The rats were subjected to the measurement protocol at the end of the ischemic period (n=3) or 15 (n=3), 30 (n=3), or 150 (n=3) minutes after it. Four rats used as untreated controls were subjected to the same manipulations as the animals receiving ischemia but without closing of the carotid snares.

Results

Choline content increased significantly in all regions during ischemia, ranging from 226% to 550% of control values (Figures 1 and 2), while the acetylcholine content decreased significantly in all regions (Figures 1 and 2).

During reperfusion, a rebound of acetylcholine levels to greater than those of the control group was observed at 15 minutes. This change reached statistical significance in the cingulate, medial frontoparietal, and lateral frontoparietal cortices (Figure 1) and the hypothalamus (Figure 2).

The choline concentration decreased progressively in all regions during recirculation. By 150 minutes, it had reached levels comparable to those of the control rats in the medial striatum, and hypothalamus, and hippocampal CA1 area (Figure 2). On the other hand, the lateral striatum and hippocampal CA2 area (Figure 2) as well as the cingulate, frontoparietal, and olfactory cortices (Figure 1) showed choline levels lower than the controls. A reciprocal relation between CBF and the choline concentration was observed when data from 30 and 150 minutes of recirculation were pooled (Figure 3, upper left panel). Regression of the choline concentration on the reciprocal of CBF yielded a β coefficient of 7.01 (SE=0.45) and a constant of 32.3 (SE=27.8), with a correlation coefficient of 0.83 (n=106, p<0.001). No correlation between CBF and the tissue choline concentration was observed at 15 minutes of reperfusion.

A significant dependence of the tissue acetylcholine concentration on the tissue choline concentration was found in the controls (slope=0.65, SE=0.24; n=68, p<0.05; Figure 3) and in the three recirculation groups pooled (slope=0.27, SE=0.06; n=135, p<0.001) but not in the ischemia group (slope=0.03, SE=0.02; n=54; Figure 3). During recirculation, CBF varied considerably (Figures 1 and 2), and this provided the opportunity to test for possible effects of CBF level (<0.3, 0.31–0.90, and >0.9
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Mean±SE BP in the groups were control, 109±1.2; ischemia, 110.7±10.7; 15 minutes recirculation, 129.5±6.4; 30 minutes recirculation, 112±2.3; and 150 minutes recirculation, 102±6.0 mm Hg. Only the 15

ml×g⁻¹×min⁻¹) on the acetylcholine–choline relation. A lack of dependence of the acetylcholine and choline concentrations was obtained in the first subgroup (slope=−0.09, SE=0.07; n=26), but significant dependence was found in the other two subgroups (slope=0.33, SE=0.09; n=75, p<0.001 and slope=0.30, SE=0.11; n=39, p<0.02, respectively; Figure 3).
Discussion

We observed considerable changes at the end of 15 minutes of forebrain ischemia in the brain tissue choline levels in all regions studied. The increase was interpreted as a lack of washout by the circulation of the choline formed from phospholipids. We have previously made similar observations in the cerebral cortex of rats subjected to permanent middle cerebral artery (MCA) occlusion. In those experiments, we were able to define a reciprocal relation between CBF and the tissue choline concentration.3 In the present experiments we obtained the same type of relation during late (30 and 150 minutes) but not early (15 minutes) recirculation. This difference may be due simply to the fact that CBF was high during early recirculation, reflecting an initial postocclusive hyperemia, and did not provide enough variation to reach the range in which the dependence of choline concentration on CBF is more pronounced. Figure 3 shows that the choline concentration varied little over the CBF range in the 15 minutes recirculation group (0.5–2.2 ml×g⁻¹×min⁻¹). After 150 minutes of recirculation, the tissue choline concentration was lower than that in the control rats in most brain regions. This observation is of interest in the context of brain choline balance following repeated ischemia and needs to be confirmed and expanded by studying the effect of repeated episodes of transient ischemia on this variable.

The acetylcholine concentration decreased during ischemia to about half of its normal value in all regions. Brain acetylcholine levels result from the dynamic equilibrium between the rate of synthesis and the rate of release with subsequent enzymatic breakdown. The two main mechanisms that could lead to a decrease in tissue acetylcholine levels are enhanced release or decreased synthesis. We have observed previously that although depressed, acetylcholine release from the cerebral cortex can still be detected during ischemia.11 Thus, decreased acetylcholine synthesis, with the consequent failure to replenish the amount lost by release and breakdown, is in all probability the mechanism that mediates the presently reported decrease in brain acetylcholine contents.

It is noteworthy that a decrease in the acetylcholine content was observed during ischemia despite a considerable excess of choline. Under normal circumstances, choline availability is rate-limiting for acetylcholine synthesis in rat brain,12,13 and hence increases in acetylcholine synthesis and content should have been obtained. The lack of such an effect reflects in all probability a decrease in the availability of the other precursor for acetylcholine synthesis,
acetyl-CoA, which originates from pyruvate,\textsuperscript{14} a product of the oxidative degradation of glucose.

Rebound of the tissue acetylcholine contents to values greater than those of the controls was observed during early recirculation. This is probably due to the fact that acetyl-CoA availability is restored more rapidly than the excess choline accumulated during ischemia can be washed away by the circulation, leading to an excess acetylcholine synthesis by a precursor-loading effect.

Dependence of the tissue acetylcholine concentration on the tissue choline level most likely reflects the fact that choline availability, as stated above, is rate-limiting for acetylcholine synthesis and thus provides an index of this process. It is interesting to note that there was no such dependence during ischemia or recirculation with CBFs of \(< 0.3 \text{ ml} \times \text{g}^{-1} \times \text{min}^{-1}\), while a significant dependence of the acetylcholine level on the choline level was again observed with CBFs of \(> 0.3 \text{ ml} \times \text{g}^{-1} \times \text{min}^{-1}\). This fact indicates a restriction of acetylcholine synthesis below a critical CBF level. The fact that no decrease in acetylcholine content is observed at 30 minutes of recirculation, when CBF in most regions is at or below this critical level, is probably due to the excess acetylcholine that accumulated during the rebound of acetylcholine synthesis during early recirculation.

Our previous observations with MCA occlusion revealed a small decrease in the tissue acetylcholine concentration in the core of the ischemic area.\textsuperscript{5} The difference between that decrease and the presently reported larger decrease in tissue acetylcholine content is probably related to the fact that CBF levels attained during four-vessel occlusion are lower than those obtained with MCA occlusion. Although we did not measure CBF during ischemia in the present experiments, data in the literature indicate that blood flow in the rat cerebral cortex is \(< 0.04 \text{ ml} \times \text{g}^{-1} \times \text{min}^{-1}\) with four-vessel occlusion\textsuperscript{9} while our previous experiments with MCA occlusion showed a minimum of \(0.2 \text{ ml} \times \text{g}^{-1} \times \text{min}^{-1}\).\textsuperscript{15}

In summary, transient forebrain ischemia 15 minutes in duration induces a decrease in the acetylcholine content of all regions studied followed by a rebound to more than control values during early recirculation and restoration of normal levels by 150 minutes of recirculation. On the basis of present and previous evidence, we hypothesize that these changes are brought about by variations in the rate of acetylcholine synthesis induced by the availability of its precursors. Dependence of the acetylcholine concentration on the choline content could be demonstrated when CBF was \(> 0.3 \text{ ml} \times \text{g}^{-1} \times \text{min}^{-1}\). The tissue choline content was reciprocally related to CBF. Tissue choline was depleted in most brain regions 150 minutes after transient ischemia. On the basis of these results, we speculate that more prolonged or repeated episodes of ischemia could lead to a greater depletion of tissue free choline, with a consequent decline in the tissue acetylcholine concentration. These phenomena could play an important role in the cognitive deficit associated with vascular dementia.

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References


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