Effects of Middle Cerebral Artery Occlusion on Cerebral Cortex Choline and Acetylcholine in Rats

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We measured regional cerebral blood flow and acetylcholine and choline concentrations in tissue fragments of normally perfused and ischemic cortical regions from 10 rats. Tissue uptake of \([^{14}C]\)iodoantipyrine was used to indicate regional cerebral blood flow, and gas chromatography–mass spectrometry was used to measure acetylcholine and choline concentrations. Cerebral cortical ischemia was induced by permanent occlusion of the middle cerebral artery, and variables were measured 2.5 or 24 hours later. A close correlation was found between tissue choline concentration and the reciprocal of regional cerebral blood flow. A large increase in tissue choline concentration was observed in the ischemic cortex. Choline production rate was estimated by plotting choline concentration against the reciprocal of regional cerebral blood flow. This rate was independent of choline concentration. Acetylcholine concentration, on the other hand, was constant in ischemic and normally perfused regions, except in the center of the ischemic region 2.5 hours after middle cerebral artery occlusion, where a significant decrease was observed. (Stroke 1989;20:1524–1530)

Acetylcholine (ACh) is a neurotransmitter of crucial significance in the physiology of the cerebral cortex. Early studies related the release of ACh from the cortical surface to physiologic events such as cortical arousal and sensory stimulation.\(^1,2\) Topical application of cholinergic agonists elicits dramatic changes in somatosensory evoked activity,\(^3,4\) and a large repertoire of effects on nerve cell discharges has been described upon microapplication of ACh in vivo.\(^3–8\) Projections from the basal forebrain account for most of the ACh present in the cerebral cortex,\(^9,10\) although intrinsic cholinergic neurons\(^11,12\) and projections from the brainstem\(^13\) have also been identified.

The analysis of ACh metabolism is complicated by the facts that its precursors also participate in numerous other reactions, there is no intermediate molecule the accumulation of which can be used to indicate ACh synthesis, and there is no distinctive breakdown product that can be used as an index of ACh release. Choline (Ch) is both a precursor and a degradation product of ACh. In addition, Ch is utilized in membrane phospholipid synthesis. The brain capacity for de novo synthesis of Ch is negligible.\(^14–17\) The sources of Ch for ACh formation are degradation of Ch-containing phospholipids within the brain itself, Ch derived from enzymatic hydrolysis of ACh released at synaptic sites, and plasma Ch.\(^16,18,19\) Ischemia may increase availability of Ch since an increase in the rate of breakdown of Ch-containing phospholipids is a known correlate of this condition.\(^20–22\) In addition, a decrease in regional cerebral blood flow (rCBF) might, in theory, increase cortical concentration of unesterified Ch since it is known that there is normally a steady output of this molecule from the brain.\(^23,24\) On the other hand, ischemia may decrease availability of the other precursor of ACh, acetylcoenzyme A (acetyl-CoA), which is generated under physiologic conditions from pyruvate.\(^16\) Thus, ischemia may exert opposing influences on the rate of ACh synthesis through alterations in the availability of its precursors.

To gain insight into these possible phenomena, we developed a methodology that permits the simultaneous determination of rCBF, Ch, and ACh in tissue fragments of the cerebral cortex of animals subjected to permanent occlusion of the middle cerebral artery (MCA). We studied short- and long-term effects of MCA occlusion on cortical rCBF, Ch, and ACh in the
normal and ischemic hemispheres of rats at various distances from the ischemic focus.

Materials and Methods

We used 10 Wistar rats weighing 250–300 g. Under halothane anesthesia, the rats were positioned on a head holder and the left MCA was exposed 3–4 mm below the temporal ridge and occluded by microbipolar coagulation. Two iliac arteries and veins were cannulated for the determination of rCBF, Ch, and ACh. After the wounds were closed and anesthesia was discontinued, the rats were placed in a restraining device designed to fit inside the animal chamber of a Biostat 5-kW microwave apparatus (Gerling Industries, Modesto, California). Each rat’s rectal temperature was maintained between 36.5° and 37.5° C with the aid of an external radiant heat source.

At 2.5 hours after discontinuation of anesthesia, the device carrying the rat was positioned inside the animal chamber of the microwave apparatus. One arterial line was used for blood sampling and the other for the continuous recording of blood pressure (BP) with a Statham P23Db strain gauge transducer (Cleveland, Ohio) connected to a Hewlett-Packard polygraph (Palo Alto, California). One venous line was used for infusion of the blood flow tracer [13C]iodoantipyrine (IAP, specific activity 50 mCi/mmol, Amersham Corp., Arlington Heights, Illinois) and the other for injection of a euthanasia agent (T-61, American Hoechst Corp., Somerville, New Jersey) to arrest the heart at the end of IAP infusion (Figure 1). IAP (65 μCi/kg body wt) was dissolved in 0.6 ml saline and injected at a rate of 1.2 ml/min over 30 seconds. At time t=28 seconds, infusion of the euthanasia agent was automatically started, and this produced cardiac arrest. An operator observed the BP and activated the microwave apparatus when BP dropped to 50 mm Hg. Microwave power (5 kW) focused to the rat’s head was then applied for 4 seconds. The rat was removed from the microwave apparatus, and the brain was removed, frozen, and dissected into regions.

Twenty samples of cerebral cortex were obtained from two coronal planes (Figure 2). Brain tissue fragments weighing 5–12 mg were homogenized in ice-cold 15% IN formic acid, 85% acetone containing deuterium-labeled internal standards for Ch and ACh assay. The homogenate was centrifuged, and the supernatant was transferred to a clean tube and extracted with dipicrylamine into methylene dichloride. Ch and ACh concentrations were measured using gas chromatography–mass spectrometry as described before.25

![Figure 1. Schematic representation of experimental setup. Rats were cannulated under halothane anesthesia, which was discontinued 2.5 hours before procedure. During that time, rats remained restrained in custom-designed device that was introduced into animal chamber of microwave apparatus shortly before tracer ([13C]iodoantipyrine, IAP C-14 or 14C-IAP) infusion. Microwave power focused to rat’s head was applied immediately after completion of tracer infusion and injection of euthanasia agent (T-61) that induced cardiac arrest. BP; blood pressure.](http://stroke.ahajournals.org/issue/C.518/1/1/1525)
rCBF was calculated from tissue and blood radioactivities using Kety's derivation of the Fick equation. 26

Results

The distribution of rCBF after 2.5 hours of MCA occlusion showed a zone of ischemia with a focus in the motor (Region 2) and somatosensory (Regions 3 and 4) areas of the neocortex in the affected hemisphere. After 24 hours of MCA occlusion, rCBF values were higher than at 2.5 hours in both hemispheres (Figures 3 and 4).

Ch concentrations were elevated two-sixfold in the ischemic compared with the contralateral (control) cortex. The highest Ch concentrations were found in the focus of ischemia (Figure 3). As with rCBF, changes in Ch concentration were less pronounced after 24 hours of MCA occlusion than after 2.5 hours (Figures 3 and 4).

Comparison of the rCBF and tissue Ch concentration data revealed what appeared to be an inverse relation, apparent not only in the areas showing the most pronounced ischemia after 2.5 hours of MCA occlusion (Figure 3) but also in moderately ischemic areas after 24 hours of MCA occlusion (Figure 4). There was also a clear tendency to such an inverse relation between tissue Ch concentration and rCBF in the nonischemic areas (Figures 3 and 4). To test the validity of this concept, tissue Ch concentration was regressed on the reciprocal of the corresponding rCBF for all regions studied. The regression analysis yielded a highly significant positive correlation ($r=0.90, p<0.001$); the intercept was 7 nmol/g and the slope was 20.8 nmol/g/min (Figure 5). We attempted to interpret the physical meaning of the parameters of this linear relation in light of the conservation principle expressed in the Fick law (Figure 5, Equation 1). Assuming that Ch exchange through brain capillaries is not a rate-limiting step, a simple model can be devised for a steady-state condition in which tissue Ch concentration is a function of arterial blood Ch concentration, the net rate of Ch production, and the reciprocal of rCBF (Figure 5, Equation 2). In this model, the intercept represents arterial Ch concentration and the slope represents the net rate of Ch production in the tissue. The experimental values obtained and shown in Figure 5 are, as will be discussed below, within the range observed by other authors with different experimental conditions and methodologies.

ACh levels did not vary between hemispheres, except in Region 3 of Plane A after 2.5 hours of MCA occlusion. This was the region with the lowest rCBF and the highest Ch concentration in the ischemic hemisphere (Figure 3).

Discussion

Occlusion of the MCA in rats is a well-established model of stroke. Various degrees of ischemia can be obtained with this model, depending on the level of occlusion and the animal strain. 27-30 Hypoperfusion of the cerebral cortex shows a gradient, from an ischemic core generally situated in the somatosensory area, decreasing in severity toward the midline and the posterior portion of the hemisphere, where anastomoses with collaterals from the anterior and posterior cerebral arteries mitigate the decrease in rCBF. 31 Events occurring in the periphery of an ischemic area, often called the penumbra, have attracted considerable attention since it is believed that the functional impairment observed in the periphery might be reversible and might therefore constitute a potential therapeutic target. The degree of cerebral ischemia generated in our experiments probably falls entirely within the definition of penumbra since it is generally accepted that infarction occurs only with an rCBF of <0.2 ml/g/min in the cerebral cortex of rats. 27 In agreement with the concepts expressed above, our results also showed
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PLANE A  PLANE B

Blood Flow

Blood Flow

FIGURE 3. Bar graphs, tissue choline and acetylcholine concentrations and regional cerebral blood flow in ischemic left (filled bars) and contralateral control (open bars) cortex of five rats subjected to permanent occlusion of middle cerebral artery 2.5 hours before. Brain regions and planes are described in Figure 2. *=p<0.05, :p<0.01 different from control by Student's paired t test. Choline concentration in Region 3 was significantly higher in Plane A than in Plane B (p<0.01).

a minimum rCBF in the somatosensory areas following MCA occlusion.

Tissue Ch concentration was linearly related to the reciprocal of rCBF. It is interesting to note that the same linear relation could be found in the core and periphery of the ischemic area, as well as at short and long intervals after MCA occlusion. There is also an indication in our data that tissue Ch concentration and rCBF might be reciprocally related even in nonischemic areas. Thus it seems that Ch, by virtue of the fact that it is produced from phospholipids at a steady rate in brain tissue and cleared by the circulation, functions as an endogenous tracer of rCBF in both normal and ischemic tissue. This property of Ch may be of practical interest, particularly if a way is found to measure brain concentrations of this molecule noninvasively by use of its characteristic nuclear magnetic resonance.

The quantitative analysis depicted in Figure 5 rests on the assumption of a rapid exchange between tissue and venous blood Ch. It has been shown that Ch exchanges between blood and tissue by either diffusion or a carrier-mediated mechanism with a very high $K_m$. Furthermore, Choi et al have shown the existence of a rapid component (half time 1.12 minutes) in the time course of the increase of brain Ch specific activity during infusion of deuterated Ch. Thus, the assumption of steady-state conditions after several hours of essentially constant blood flow, as in our experiments, seems justified. The fact that the rate of Ch production, estimated by the slope of tissue Ch concentration versus the reciprocal of rCBF, is constant (a straight line) over a wide range of tissue Ch concentrations suggests a zero-order process. This is precisely what several laboratories have previously shown, studying this phenomenon by incubating isolated brains following decapitation. The net rate of brain Ch production, estimated from the arterio-venous difference for this molecule, has been reported to be between 4 and 10 nmol/g/min. These values, as well as those for Ch production in incubated brains mentioned above, represent the average of the entire organ. Our estimate, on the other hand, represents a single region (the cerebral cortex). The discrepancies between estimates of this variable may therefore be due to regional variations about which there is presently a lack of detailed information.

Tissue ACh concentration, on the other hand, was remarkably constant throughout the entire neocortex. Only the ischemic core showed an ACh level lower than normal. It is important to note that ACh is synthesized by a reversible reaction, catalyzed by choline acetyltransferase, with acetyl-CoA and Ch as precursors. The concentration of reac-
In conclusion, simultaneous measurement of rCBF and tissue Ch and ACh concentrations in ischemic and normally perfused cortical regions has revealed a close relation between tissue Ch concentration and rCBF that allows estimation of the net rate of Ch production in the cerebral cortex of conscious rats. Tissue ACh concentration remained constant except at the core of the ischemic area.

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**Figure 5.** Least-squares regression of tissue choline (Ch) concentration and reciprocal of corresponding regional cerebral blood flow (F) for all five regions and both planes 2.5 (•) and 24 (□) hours after middle cerebral artery occlusion in rats (n=40). Every point represents mean of five rats. Correlation coefficient (r) was significant at p<0.001. Insert: Theoretical model of Ch exchange between brain tissue and blood. In steady-state conditions, it can be assumed that net rate of Ch production by brain (Ch) equals product of F times difference between arterial (Ch_a) and cerebral venous (Ch_v) Ch concentrations (Equation 1). Assuming diffusion equilibrium between tissue and cerebral venous blood of particular region, steady-state tissue Ch concentration (Ch_t) is related to Ch_a, Ch_v and F by Equation 2.


**KEY WORDS** • cerebral blood flow • neurotransmitters • rats
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