Metabolic Alterations in Rabbit Cerebral Arteries Caused by Subarachnoid Hemorrhage

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The effect of subarachnoid hemorrhage on metabolic rates in rabbit cerebral arteries was investigated by measuring adenosine triphosphate (ATP) content and L-lactate release. The mean ± SEM ATP content was 0.38 ± 0.02 μmol/g wet wt in control rabbit basilar arteries (n = 6). The ATP content decreased significantly to 0.17 ± 0.02 μmol/g wet wt 2 days after experimental subarachnoid hemorrhage (n = 6), although only a slight decrease was detected in the basilar arteries 2 days after cisternal injection of the same amount of artificial cerebrospinal fluid. Hypoxia significantly decreased ATP content in the control basilar arteries to 0.26 ± 0.04 μmol/g wet wt (n = 6). The same degree of hypoxia did not decrease ATP content in the basilar arteries after subarachnoid hemorrhage. Release of L-lactate was significantly higher from the arteries after subarachnoid hemorrhage than from the control arteries under both aerobic and hypoxic conditions. Our results indicate that subarachnoid hemorrhage induced an alteration of metabolic rates in rabbit cerebral arteries. The oxygen-requiring pathways to synthesize ATP may be important in control cerebral arteries; however, after experimental subarachnoid hemorrhage, the main pathway in the cerebral arteries may shift from oxygen-requiring pathways to an anaerobic glycolytic pathway. (Stroke 1988;19:883-887)

In guinea pig teniae coli and dog tracheal muscle, it has been reported that hypoxia and/or depletion of glucose induced prolonged constriction of the smooth muscle. The constriction under these circumstances was characterized to be a rigor since it was induced by metabolic depletion accompanied by a decreased concentration of adenosine triphosphate (ATP) and was insensitive to chelation of calcium. The metabolic impairment in cerebrovascular smooth muscle cells may be related to the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage (SAH). However, the influence of SAH on metabolism of the major cerebral arteries has not been studied sufficiently. A previous study by Vinall and Simeone produced rigor in the cerebral arteries in vitro in an attempt to clarify the pathophysiology of cerebral vasospasm after SAH. However, the attempt was unsuccessful. The contractile responses to metabolic depletion of the cerebral arteries may be different from that of teniae coli and tracheal smooth muscle.

Materials and Methods

Animal preparations. Male New Zealand White rabbits weighing 2.9–3.4 kg were anesthetized with an intramuscular injection of a mixture of 20 mg/kg ketamine, 5 mg/kg xylazine, and 0.25 mg/kg acepromazine. The rabbits were intubated and were paralyzed with an intravenous injection of 0.08
mg/kg pancuronium bromide. Ventilation was maintained with a Harvard dual-phase-control respirator (South Natick, Massachusetts). In each rabbit, the left ear artery was cannulated for monitoring blood pressure and for withdrawing arterial blood. A 23-gauge butterfly needle was inserted percutaneously into the cisterna magna and connected to a three-way stopcock. The outlet was used for injection of arterial blood or artificial cerebrospinal fluid (CSF) of the following gram-per-liter composition: NaCl 7.3, NaHCO3 2.1, CaCl2 0.278, MgCl2 0.244, KCl 0.224, glucose 0.666, and urea 0.360. SAH was produced in six rabbits by injecting 5 ml fresh, autologous, nonheparinized arterial blood into the cisterna magna. Six rabbits received artificial CSF, and six rabbits received no intracisternal injection of blood or CSF (controls).

Artery preparation and tension recording. Each rabbit was reanesthetized with 60 mg/kg i.m. ketamine and exsanguinated from the femoral artery. The brain, with the basilar artery in situ, was removed and placed in a dissecting chamber filled with Krebs solution (millimolar composition: NaCl 120, KCl 4.5, MgSO4 10, NaHCO3 27.0, KH2PO4 1.0, CaCl2 2.5, and dextrose 10.0). The basilar arteries were dissected free under magnification, and 3-mm-long arterial rings were prepared. The specimen was then suspended between two L-shaped rods in an organ bath with a 10 ml working volume, which was gassed with 95% O2 and 5% CO2. pH of the solution ranged from 7.40 to 7.50; resting tension was adjusted to 400 mg. Isometric tension was recorded using a Grass FT.03 force-displacement transducer (Quincy, Massachusetts) and was displayed on a Soltec 3418 chart recorder (San Fernando, California). After 60 minutes of aerobic equilibration, hypoxia was induced by changing to a gas mixture containing 95% N2-5% CO2 15 minutes before the application of 40 mM KCl under aerobic conditions. *p<0.01. Therefore, an extraction period of 5 minutes was employed, and the amount of ATP liberated was regarded as the ATP content of the muscle.

Assay of lactate release. The amount of lactate released from the muscle was determined by a reaction of lactate dehydrogenase. Vascular muscle (approximately 5 mg) was incubated in 1 ml of Krebs solution under aerobic or hypoxic conditions for 60 minutes at 37° C after equilibration in Krebs solution for 60 minutes. Then, 0.4 ml solution was treated with lactate dehydrogenase to obtain reduced nicotinamide adenine dinucleotide (NADH). The amount of NADH was determined by means of its absorbance at 340 nm.

Statistical analysis. The data were expressed as mean ± standard error of the mean (SEM). Multiple comparisons of ATP contents in the basilar or middle cerebral arteries of the control group, the artificial CSF group, and the SAH group were evaluated using Scheffé's test after analysis of variance (ANOVA). Multiple comparisons of ATP contents in the basilar arteries with 40 mM KCl were evaluated also using Scheffé's test after ANOVA. The other comparisons were evaluated using Student's t test. When p was <0.01, the values were considered to be significantly different.

Drugs. We used ATP and luciferin-luciferase reagent from Sigma Chemical Co., St. Louis, Missouri, and L-lactate dehydrogenase, glutamate-pyruvate transaminase, β-NAD, and L-glutamic acid from Boehringer, Mannheim, FRG. All other chemicals were of reagent grade or the purest grade commercially available.
Results

Effect of hypoxia on KCl-induced tension. Figure 1 shows the time course of KCl-induced tension in control rabbit cerebral arteries and in arteries 2 days after experimental SAH under aerobic and hypoxic conditions. When control cerebral arteries were exposed to a high concentration (40 mM) of KCl under aerobic conditions, they developed a rapid phasic contraction followed by a sustained tonic contraction. The tonic contraction continued for >7 minutes. No significant difference in the phasic and tonic components was detected between the control and SAH groups. Under hypoxia, 40 mM KCl induced transient phasic contractions of normal size and small tonic contractions in control rabbit cerebral arteries. Hypoxia also decreased the magnitude of tonic contractions in cerebral arteries after SAH. However, the tonic contractions of the arteries after SAH were significantly greater than those of control arteries under hypoxia.

ATP content in rabbit cerebral arteries. Figure 2 shows the ATP content in rabbit basilar and middle cerebral arteries. Control ATP contents were 0.38 ± 0.04 μmol/g wet wt in basilar arteries and 0.22 ± 0.03 μmol/g wet wt in middle cerebral arteries. ATP content in basilar arteries decreased significantly to 0.17 ± 0.02 μmol/g wet wt 2 days after SAH, although only a slight decrease, to 0.32 ± 0.05 μmol/g wet wt, was detected 2 days after cisternal injection of artificial CSF. ATP content in middle cerebral arteries was not influenced significantly 2 days after SAH (0.18 ± 0.03 μmol/g wet wt) nor 2 days after cisternal injection of artificial CSF (0.25 ± 0.02 μmol/g wet wt). Contractions induced by 30 minutes' incubation with 40 mM KCl under aerobic conditions did not significantly influence the ATP content of either control (0.34 ± 0.02 μmol/g wet wt) or SAH arteries (0.18 ± 0.03 μmol/g wet wt).

Effect of hypoxia on ATP content. Figure 3 shows the effect of hypoxia on ATP content in control basilar arteries and basilar arteries 2 days after SAH. Hypoxia significantly decreased ATP content in control basilar arteries, to 0.24 ± 0.02 μmol/g wet wt in 10 minutes and 0.26 ± 0.02 μmol/g wet wt in 30 minutes. The same degree of hypoxia did not reduce ATP content significantly in basilar arteries 2 days after SAH.

L-Lactate release from basilar arteries. Under aerobic conditions, significantly more L-lactate was released from basilar arteries (Figure 4) in the SAH group (5.4 ± 0.4 μg/mg wet wt/hr) than in the control group (3.4 ± 0.4 μg/mg wet wt/hr). Hypoxia increased the amount of L-lactate released from both control and SAH arteries, though the amount in the SAH group (9.5 ± 0.8 μg/mg wet wt/hr) was significantly greater than that in the control group (5.8 ± 0.5 μg/mg wet wt/hr).

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Discussion

We characterized smooth muscle cell metabolism in the rabbit cerebral arteries under both aerobic and hypoxic conditions. The changes of ATP content in and L-lactate release from the arteries after intracisternal injection of blood suggested that SAH induced metabolic alterations in the arteries. Three major pathways to produce ATP in arterial smooth muscle are known: oxidative phosphorylation, anaerobic glycolytic pathways, and β-oxidation of fatty acids. The relative importance of these three pathways in physiologic or pathologic conditions may be different in different arteries. Cook et al., using a histochemical technique, reported that oxidative pathways were important in small coronary arteries, though in large coronary arteries the anaerobic glycolytic pathway played the major role. Since both oxidative phosphorylation and β-oxidation of lipids require oxygen and the anaerobic glycolytic pathway does not, the relative importance of these pathways can be in part decided by the responses of the arteries to hypoxia. It has been reported that a reduction of oxygen tension in the incubation medium decreased creatine phosphate and ATP levels and decreased the magnitude of tonic contractions induced by high concentrations of KCl in arterial smooth muscles. On the other hand, it has also been reported that hypoxia did not influence ATP content or KCl-induced contractions of arterial smooth muscles. These different responses of vascular smooth muscle to hypoxia seem to be caused by the relative importance of the metabolic pathways in each artery, as well as by the experimental conditions. Vinall and Simeone have demonstrated that contractile responses of bovine middle cerebral arteries to serotonin or whole blood were significantly depressed after incubation under hypoxia. Our findings on the reactivity of rabbit basilar arteries to KCl were basically consistent with those findings. It has been reported that cerebral arteries might be among the arteries most sensitive to hypoxia since ATP content in cerebral arteries decreased more quickly than in extracranial arteries after incubation under hypoxia. We found that ATP content in rabbit cerebral arteries was similar to that in dog cerebral arteries reported by Kirsch et al., who measured ATP content using the microchemical methods of Lowry. We also confirmed that hypoxia significantly decreased ATP content within 10 minutes and significantly decreased the tonic contraction induced by high concentrations of KCl. Therefore, oxygen-requiring pathways to produce ATP may play an important role in normal rabbit cerebral arteries. On the other hand, it seems that there also exists an anaerobic pathway to produce ATP even under aerobic conditions since L-lactate was released from the arteries under aerobic conditions. The decrease in ATP content induced by hypoxia was roughly 50% of that under aerobic conditions, and in spite of additional incubation under hypoxia for 30 minutes, ATP content did not decrease further. The existence of an anaerobic glycolytic pathway and quick activation of this pathway under hypoxia may be reasons why hypoxia cannot induce rigorlike contraction of the cerebral arteries.

It has been reported that ATP content in arterial smooth muscle was not influenced by continuous constriction induced by high concentrations of KCl but only by metabolic impairments. We also found that incubation with high concentrations of KCl did not induce a significant decrease in ATP in the cerebral arteries, so the decrease in ATP in the basilar arteries after SAH may be due to impairment of metabolism rather than to persistent constriction. Our study showed that more L-lactate was released from arteries after SAH than from control arteries and that the contractile response of the arteries after SAH was less sensitive to hypoxia than that of control arteries. Our data suggest that the main pathway to produce ATP may shift from oxygen-requiring pathways to an anaerobic glycolytic pathway after SAH. Blood in the subarachnoid space may have some direct effect on metabolism of the cerebral arteries. An acute increase of intracranial pressure in this model did not have a significant effect on metabolism since injection of the same volume of artificial CSF into the cisterna magna did not significantly influence the ATP content of either basilar or middle cerebral arteries. However, experimental SAH resulted in a significant decrease in ATP in the basilar artery while affecting no change in the middle cerebral artery. After SAH was induced by injecting blood into the cisterna magna, thick subarachnoid clots were observed around the basilar arteries but not around the middle cerebral arteries. Only the basilar arteries are likely to be under continuous hypoxia as a result of clogging of the adventitial layer by thick clots. Because the cerebral arteries lack vasa vasorum, clots in the subarachnoid space are likely to induce hypoxia. Thickening of cerebral endothelial cells can also disturb oxygen supply to the smooth muscle from the intraluminal side of the arteries. Moreover, vasocostriction itself by vasoactive substances released from surrounding clots and impairment of vasodilatation after SAH may decrease the blood supply, accordingly inducing hypoxic stress in the smooth muscle. As a result, continuous hypoxia may shift the main metabolic pathway from oxygen-requiring ones to anaerobic glycolysis. Such metabolic alterations in the cerebral arteries could play an important role in the genesis of delayed vasospasm after SAH.

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