Effect of Acute and Chronic Reduction of Cerebral Blood Flow on Glucose Metabolite Levels in SHRSP

YASUO NARA, PH.D., RYOICHI HORIE, M.D., AND YUKIO YAMORI, M.D.

SUMMARY Glucose metabolites in the rat brain were measured under various stages of ischemia. In acute ischemia induced by bilateral carotid artery ligation, phosphocreatine and ATP levels were significantly decreased and lactate levels were significantly increased in stroke-prone spontaneously hypertensive rats (SHRSP). These extreme changes were not observed in normotensive Wistar-Kyoto rats (WKY) and stroke-resistant spontaneously hypertensive rats (SHRSR), under the same conditions. In chronic ischemia induced by long-lasting regional cerebral blood flow reduction due to severe hypertension, similar changes were observed only in SHRSP at the advanced stage. The levels of glucose metabolites in the brain were confirmed to be well maintained within control ranges even though the cerebral tissues were subjected to the chronic ischemia related to severe hypertension.

SINCE stroke-prone spontaneously hypertensive rats (SHRSP) were established by selection from among spontaneously hypertensive rats (SHR) by Yamori et al.,¹, ² the mechanisms of severe hypertension and stroke are now better understood.³, ⁴ These animal models have various similarities to human diseases and can be used as models for studying the pathogenesis of cerebrovascular diseases in man.⁵ Previously, Yamori⁶ reported that regional cerebral blood flow (rCBF) in the brain was actually decreased in the SHRSP when the blood pressure surpassed 200 mm Hg, despite the generally held theory that an increase occurred due to "hypertension breakthrough." When the brain is maintained under conditions of chronic mild ischemia, the metabolism in the brain is, as expected, disturbed. As glucose is the main energy source for the brain, alterations in glucose metabolites during various stages of ischemia are good indicators of cerebral metabolism.

We measured the metabolites in the brains of adult, old non-symptomatic and old symptomatic SHRSP, and also in the brains of WKY, SHRSR and SHRSP under conditions of acute ischemia 2 hours after bilateral carotid artery ligation.

Materials and Methods

In acute experiments, bilateral carotid arteries were ligated (under pentobarbital (60 mg/kg, i.p.) anesthesia) in 3-month-old male WKY, SHRSR and SHRSP, 5 in each group. Two hours after the ligation they were immersed in dry ice-acetone (—75°C).

In chronic experiments, male WKY, SHRSR and SHRSP, 5 in each group, with or without stroke, were used. Symptomatic diagnosis of stroke in SHRSP was made according to previously reported criteria.⁷ Animals which became akinetic, lethargic and hyporesponsive to painful or arousal stimuli were believed to have stroke. These rats were kept free from stress without anesthesia and the whole body was immediately immersed in dry ice-acetone to measure metabolic intermediates of the brains.

After complete freezing, the forebrains of these rats were quickly removed and tissue extract from the brains prepared in a cold room (—20°C) by the method of Folbergrova et al.⁸ Metabolic intermediates, ATP, phosphocreatine, pyruvate, lactate, and glucose, were determined enzymatically by the fluorometric method.⁹ Blood pressure was measured by an indirect tail-pulse-pickup method,¹⁰ and rCBF was measured by a hydrogen clearance method in non-restrained conscious rats kept in a small gas chamber. In order to avoid the effect of implanted electrodes, age-matched groups of male rats, 5 from each of WKY, SHRSR and SHRSP strains, were used only for rCBF measurement. The measurement of rCBF, which was described in detail in our previous report,¹¹ was started 2 weeks after the implantation of the electrodes into the frontal cerebral cortex.

Results

1) Acute Experiments

All of the 3-month-old SHRSR and SHRSP developed hypertension 180 ± 4 and 200 ± 4 mm Hg, respectively, while the WKY remained normotensive (120 ± 3). Blood flow in the brain was decreased in the SHRSP (63.6 ± 5.8 ml/min/100 g) but was not changed in the WKY (101.0 ± 7.4 ml/min/100 g) and was slightly increased in the SHRSR (110.9 ± 6.4 ml/min/100 g). Acute ischemia in the brain, as induced by bilateral carotid ligation, was severe only in the SHRSP. rCBF, 2 hours after the ligation, was not detected in SHRSP but was 10–20 ml/min per 100 g in SHRSR and WKY. As shown in figure 1, ATP, phosphocreatine, and pyruvate levels were significantly decreased, and the lactate level was significantly increased only in SHRSP. In the SHRSR and WKY rats the levels of lactate were slightly increased following ligation. SHRSP were the most sensitive to acutely induced ischemia.
2) Chronic Observation

Figure 2 shows glucose metabolites in WKY, SHRSR and SHRSP rats at the ages of 3 and 10 months. There were no marked alterations of cerebral glucose metabolism in WKY, SHRSR and SHRSP without symptoms of stroke at the age of 3 and 10 months. ATP and phosphocreatine levels were significantly decreased and lactate levels were increased in SHRSP with symptoms of stroke when compared to the age-matched asymptomatic SHRSP.

Discussion

The oxygen supplied to the brain is consumed in metabolism of glucose so that under conditions of a limited oxygen supply, glucose metabolism is disturbed and brain function is affected. When the supply of oxygen was depleted following bilateral ligation of carotid arteries, extreme metabolic disorder was observed only in the SHRSP. Marked reduction of rCBF was noted only in SHRSP after bilateral carotid ligation. These findings indicate that hypertensive structural vascular alterations, which are more severe in SHRSP with severe hypertension, affect brain perfusion through the vertebro-basilar system after bilateral carotid ligation. The observation is supported by previous work which showed marked alterations in catecholamine fluorescence in the brains of SHRSP but not of SHR and WKY rats after bilateral carotid ligation.11

Fujishima et al.18 and Iguchi et al.19 reported that glucose metabolism in WKY and SHR was affected by ligation and the metabolism disorder was more severe in SHR than in the WKY. In these studies SHR did not show a marked difference following ligation. This discrepancy can probably be attributed to the age of the animals. We used 3-month-old rats while they used 5 to 9-month-old rats which, in general, had had structural vascular changes. When blood pressure in SHRSP rises over 200 mm Hg, rCBF is remarkably decreased. During ischemia induced by chronic reduction of rCBF, blood vessel reactions in the brain are changed in two stages.4 The first stage involves functional rCBF reduction. rCBF can recover to its original level by antihypertensive treatment or can be increased in response to CO2 inhalation. The second is the organic phase of rCBF reduction, where rCBF cannot be reversed with antihypertensive treatment nor does it increase with CO2 inhalation. In SHRSP, the first occurs at about 3 months and the second stage at 10-months. Even though brain blood vessels change through these 2

Metabolic Intermediate Levels in Rat Brains (Forebrain)

![Figure 1. Levels of glucose metabolites in the brain after bilateral carotid artery ligation. **: Significant difference from SHRSR and WKY (p < 0.01).](image1)

![Figure 2. Metabolic intermediate levels in rat forebrain SHRSP with stroke.](image2)
stages with chronic ischemia, the metabolism of glucose was maintained constant until stroke occurred.

Bilateral carotid ligation is generally regarded as an effective experimental method to produce ischemia but the present experiment showed that such an approach could not simulate the ischemia which spontaneously develops with severe hypertension. Enzymes of glucose metabolism adapt fairly well to the unfavorable condition so that the levels of metabolites in the brain are maintained within a normal range even though the brain is exposed to conditions of chronic mild ischemia. Therefore, chronic mild ischemia may not directly induce metabolic damages in the brain but rather accelerates cerebrovascular damage by increasing permeability of endothelial cells and/or by accelerating medial degeneration and finally causes arterionecrosis, the basic process of cerebral hemorrhage and infarction, that is, arterionecro-thrombotic stroke. Only in these advanced stages with vascular lesions, does cerebral metabolism seem to be greatly disturbed, secondarily to vascular damages. Thus, the present observations confirm that with stroke in SHRSP, the pathogenic changes in the cerebrum are of vasogenic origin.

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Y Nara, R Horie and Y Yamori

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