S-Adenosyl-L-Methionine Ameliorates Ischemic Brain Metabolism in Spontaneously Hypertensive Rats

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Abstract—Effects of S-adenosyl-L-methionine (SAM) on the metabolism in ischemic brain were investigated. The ischemic model employed was an incomplete cerebral ischemia of the spontaneously hypertensive rat (SHR) produced by the occlusion of both common carotid arteries. One hundred mg/kg of SAM was administered (i.p.) 6 times from the beginning of occlusion at 30 min intervals. At 3 hr after the onset of occlusion, animals were killed by microwave irradiation and creatine phosphate (CrP), ATP, glucose, lactate and γ-aminobutyric acid (GABA) contents in the brain were measured. SAM significantly mitigated both the reductions in CrP, ATP and glucose levels and the increase in GABA level due to the cerebral ischemia. In another set of experiments with the same experimental schedule, water content in the brain was examined. SAM significantly suppressed the increase in water content due to the cerebral ischemia. These results indicate ameliorating effects of SAM on the metabolism in ischemic brain.

It has been reported that SAM has beneficial effects on ischemic brain; SAM suppressed brain edema (1) and prevented neuronal death in hippocampus CA-1 (2) in ischemic brain, using a stable salt of SAM, SAM-2 sulfate·tosylate (3). In the above studies, mainly near-complete ischemia models with succeeding recirculation of the gerbil (4, 5) or rat (6) were employed. When some compound is found to have a desirable effect on damaged brain, it is always important to know how brain metabolism is affected by the compound. However, in the brain which is subjected to complete or near-complete ischemia for a relatively short period with succeeding recirculation, metabolism, especially energy metabolism, recovers fairly rapidly (7–9), and such models may be improper for studying the effects of drugs on the metabolism. Thus, in the present study, in order to examine the effects of SAM in the metabolism of ischemic brain, we have used an incomplete permanent ischemia model of SHR produced by the occlusion of both carotid arteries (10–13). Following bilateral carotid occlusion, relatively severe ischemia of the brain develops in SHR, since the lower blood pressure limit of cerebral autoregulation is shifted to a higher level in SHR than in normotensive rat (14) and also since the cerebral perfusion pressure might be lowered to a greater extent in SHR than in normotensive rat (15). In this model, either a reduction in ATP or an increase in lactate level lasts for hours or even days (16, 17).

In the present study, effects of SAM on energy metabolite contents, the content of the neurotransmitter GABA as well as brain edema in ischemic brain were examined, and the beneficial effects of SAM were observed.

Materials and Methods
Chemicals: One hundred mg/kg as the free form of SAM or vehicle was administered intraperitoneally (i.p.) to animals 6 times from the beginning of occlusion at 30 min intervals. SAM was repeatedly administered because of its short life time in the body (18). The actual form of the compound used was SAM-2 sulfate·tosylate with mannitol (Fuji Chemical Industry Co., Ltd.). One hundred mg SAM is contained in 312 mg of...
the compound (192 mg SAM-2 sulfate-tosylate + 120 mg mannitol). Mannitol is added in this compound as a filler. Since the ineffec-tiveness of mannitol contained in the compound was fully demonstrated previously (1), no special attention was paid to mannitol in this study. The compound was dissolved in disodium phosphate solution adjusted to pH 5.7.

Incomplete cerebral ischemia: Male SHRs, 19 weeks old and 300–380 g in weight, were used. Operation was performed under light halothane anesthesia. Both common carotid arteries were isolated and permanently occluded with sutures. Immediately after the onset of ischemia, halothane anesthesia was turned off, and ischemia was continued for 3 hr.

Determination of brain energy metabolites and GABA: At 3 hr after the onset of occlusion (at 30 min after the last injection), animals were killed by microwave irradiation (Shin-nihonmusen, NJE2601; 4 kW, 3.0 sec), and the supratentorial portion of the brain was taken out. Animals in the normal group were killed by microwave irradiation without any treatment beforehand. The cortex (Cx) and the remaining portion which is called the subcortex (S) in this paper, were homogenized in 15 ml and 10 ml of 0.5 M perchloric acid with 1 mM EDTA, respectively. Homogenates were centrifuged at 3,000 g for 10 min, and 5 ml supernatants were neutralized with 1.6 ml 2 M KHCO₃. Mixtures were centrifuged at 1,500 g for 10 min, and the supernatants were at -80°C until assayed. CrP, ATP, glucose and lactate were assayed enzymatically (19). GABA was assayed according to the method of Okada et al. (20).

Determination of brain water content: At 3 hr after the onset of occlusion, animals were decapitated and the supratentorial portion was rapidly taken out. Animals in the normal group were decapitated without any treatment beforehand. The cortex and subcortex of each animal were placed onto aluminum cups separately and weighed (wet weight). After the brain tissues were completely dried in an oven at about 100°C for 48 hr, they were weighed again (dry weight). Water content was calculated as a percentage of wet weight according to the following equation:

\[ \text{H₂O (\%) = (wet weight - dry weight)/wet weight} \times 100 \]

Determination of blood glucose: Blood was taken from a tail vein, and blood glucose level was measured by a YSI Model 23A Glucose Analyzer (Yellow Spring Instruments Inc.). Statistical analyses: Statistical comparisons were performed with Mann-Whitney’s U-test or Student’s t-test.

Results

Effects of SAM on energy metabolite contents in ischemic brain: Figure 1 shows CrP, ATP, glucose and lactate contents of the normal (N), ischemic control (C) and SAM-treated (SAM) groups. The mean values of CrP in the normal group were 5.34 μmol/g (Cx) and 5.84 μmol/g (S), respectively. The corresponding values in the ischemic control group were remarkably reduced. Although there was no significant difference of CrP level in the cortex between the ischemic control and SAM-treated groups, the reduction in CrP due to the ischemia in the subcortex was significantly mitigated in the SAM treated group. In the cortex, the mean ATP value was 3.05 μmol/g in the normal group and was reduced to almost 1/10 in the ischemic control group. In the SAM-treated group, the mean ATP value was about half of that in the normal group and 4.3 times larger than that in the ischemic control group, indicating a remarkable prevention of the reduction in ATP due to ischemia. In the subcortex, the mean ATP value was 3.02 μmol/g in the normal group and was reduced to about 1/10 in the ischemic control group. In the SAM-treated group, the mean ATP value was about half of that in the normal group and 4.3 times larger than that in the ischemic control group, indicating a remarkable prevention of the reduction in ATP due to ischemia. In the subcortex, the mean ATP value was 3.02 μmol/g in the normal group and was reduced to about 40% in the ischemic control group. In the SAM-treated group, the mean ATP value was about 80% of that in the normal group and about twice of that in the ischemic control group, showing a remarkable prevention of the reduction in ATP, like in the cortex. In the cortex, the mean glucose value was 1.40 μmol/g in the normal group and was reduced to about 1/4 in the ischemic control group. In the SAM-treated group, the mean glucose value was increased to about a 1.5 times higher level than the normal group. However, there was a fairly large variability in individual values. Most glucose values in the SAM-treated group were either larger or
smaller than the normal range. In the subcortex, the mean glucose value in the normal group was 1.23 μmol/g and was very close to that in the ischemic control group. In the SAM-treated group, the mean glucose value was quite large, being significantly larger than both the normal and ischemic control groups. The mean lactate values in the normal group were 1.83 μmol/g (Cx) and 1.05 μmol/g (S), respectively. Those values in the ischemic control group and in the SAM-treated group were remarkably increased, and there were no significantly differences between them.

Effects of SAM on GABA content in ischemic brain: Figure 2 shows GABA contents in each group. The mean values of GABA in the normal group were 1.64 μmol/g (Cx) and 1.95 μmol/g (S), respectively. GABA levels in the ischemic control group were increased to 2.5 times (Cx) and 2.2 times (S) as much as the respective normal values. In the SAM-treated group, the mean values of GABA were significantly reduced in both regions from the ischemic control level.

Fig. 1. Creatine phosphate (CrP), ATP, glucose (Glu) and lactate (Lac) contents in the cortex (Cx) and subcortex (S) of the normal (N), ischemic control (C) and SAM treated (SAM) groups of SHR. The duration of ischemia was 3 hr. 100 mg/kg SAM or vehicle was administered (i.p.) 6 times from the beginning of occlusion at 30 min intervals. Abbreviations and the experimental conditions are the same in succeeding figures. Each column shows the mean±S.E.M. Number of animals: N(5), C(10), SAM(10). Significant difference between the SAM-treated and ischemic control groups (U-test), **P<0.025, ***P<0.01, ****P<0.001.

Fig. 2. GABA contents in the cortex and subcortex of the normal (N), ischemic control (C) and SAM-treated (SAM) groups. Each column shows the mean±S.E.M. Number of animals: N(5), C(10), SAM(10). Significant difference between the SAM-treated and ischemic control groups (U-test), **P<0.025, ****P<0.001.

Taking the difference between the mean value of the ischemic control group and that of the normal group as 100%, in the SAM-treated group, the mean values were 49% (Cx) and
Effects of SAM on water content in ischemic brain: Figure 3 shows the water content of the normal, ischemic control and SAM-treated groups. Mean values of the water content in the normal group were 78.7% (Cx) and 76.4% (S), respectively; and the values in the ischemic control group were significantly increased to 80.3% (Cx) and 77.8% (S). In the SAM-treated group, water contents were significantly lower than the respective values in the ischemic control group. Furthermore, there was no significant difference of water content in the subcortex between the normal and SAM-treated groups.

Effects of SAM on blood glucose content in either normal or ischemic brain: Since the SAM-treated group had higher glucose values than normal ones in both the cortex and subcortex, effects of SAM on blood glucose level were examined. Figure 4A shows the results of administering SAM or vehicle 6 times at 30 min intervals to normal rats. There were no noticeable changes during 3 hr both in the vehicle- and in the SAM-treated groups. Figure 4B shows the results when SAM or vehicle was injected to animals subjected to carotid artery occlusion with the same schedule as in the former experiments. In this case, blood glucose values were continuously increased until the end of 3 hr ischemia in both groups and reached a level of around 150% of the normal value. There was no noticeable difference between the vehicle- and SAM-treated groups. Thus, although an increase in blood glucose value due to ischemia was observed, no specific increase in the SAM-treated group was found.

Discussion
In the present study, the effects of SAM
on changes in energy metabolites, the neurotransmitter GABA, and water content in the ischemic brain were investigated, using a bilateral common carotid artery occlusion model in SHR. This model is an incomplete ischemia because there is substantial remaining cerebral blood flow through the verteobasilar circulation. In this model, reduction in blood flow during ischemia is severe in the cerebral cortex and is relatively mild in the subcortex (21, 22). Correspondingly, the reductions in CrP, ATP and glucose during ischemia were severe in the cortex and mild in the subcortex (Fig. 1). In the cortex of many animals in the ischemic control group, both ATP and glucose levels were close to depletion. It is obvious that this status is exceedingly dangerous for maintenance of normal brain functions and structures. It was clearly demonstrated that SAM mitigated or prevented the reduction due to the incomplete ischemia in high-energy phosphates, CrP and ATP, and in glucose, the major substrate for energy production in the brain, as well. These results mean that SAM ameliorated the energy metabolism in the incomplete ischemic brain.

It has been reported that there is an increase in GABA content in ischemic brain (23–25). The present study demonstrated that SAM prevented the increase in GABA content in the incomplete ischemic brain. This means that SAM ameliorated the disturbed metabolism of GABA in the incomplete ischemic brain.

From the above results, it can be concluded that SAM has beneficial effects upon brain metabolism, which is critically disturbed due to ischemia.

Glucose content in the ischemic brain was markedly increased by SAM treatment without a concomitant specific increase in blood glucose content in the SAM-treated group. Since some animals in the ischemic control group showed larger values of glucose than the normal range in the subcortex, it appears that when damage due to the incomplete ischemia is relatively mild, there might be a status in the ischemic brain in which glucose level exceeds the normal range. Accordingly, it is possible to interpret that in the case of the SAM-treated group, even in the cortex, glucose content exceeded the normal range, owing to prevention of ischemic damage (see below).

The prevention of ATP reduction in ischemic brain could be, in the case of incomplete ischemia, either due to a reduction of the overall metabolic rate in the brain, due to an amelioration of cerebral metabolism by a direct action on cerebral parenchyma, or due to an indirect amelioration of cerebral metabolism through the increase of lowered cerebral blood flow. With regard to the first possibility, it is unlikely that both high-energy phosphates and glucose are maintained at a high level after a long ischemic interval, 3 hr, in the present study, even with reduction of overall metabolic rate. SAM is involved in the reaction of membrane phosphatidylcholine formation from phosphatidylethanolamine as a methyl donor (26–28). This reaction might have an important role in preventing ischemic damage, since it has been known that phospholipids metabolism is perturbed in ischemic brain (29–31) and also because the membrane fluidity of erythrocytes is increased by methylation of phosphatidylethanolamine (32). The second possibility of direct action on cerebral parenchyma has been supported by Trovarelli et al. (31), who demonstrated that SAM prevented changes in choline lipids in ischemic brain. Considering the fact that in their study, intraperitoneally administered SAM (200 mg/kg) was significantly incorporated into the gerbil brain and in that situation the reduction in phosphatidylcholine in the brain due to ischemia was significantly mitigated, it is likely that a part of repeatedly administered SAM (100 mg/kg, i.p.) in the present study might be incorporated into the SHR brain and exert a protective action on membrane lipids even with the reduced blood flow. The third possibility of indirect amelioration of cerebral metabolism through the increase of lowered cerebral blood flow could occur as follows: In the SHR brain that was subjected to bilateral carotid artery occlusion for several hours, blood flows in the cerebral cortex and in one of the subcortical regions, the thalamus, were measured; and they were decreased to about 9% and 17% of the normal values, respectively (22). It has been already reported that SAM
has an ameliorating effect on erythrocyte deformability (33), presumably through an increase of membrane fluidity. Since reduced cerebral blood flow in the ischemic SHR brain could be expected to be ameliorated by the above effect of SAM which is abundantly present in the circulatory system after injection, it is highly probable that a presumed increase in cerebral blood flow with SAM could cause in turn the amelioration of metabolism in the ischemic brain due to an improved supply of both glucose and oxygen and also due to normalization of membrane phospholipids (31) with a larger amount of SAM which could be incorporated into the brain. The increased glucose content both in the cortex and subcortex in the SAM-treated group to a level even above the normal range supports the third possibility because if reduced cerebral blood flow is ameliorated in ischemic brain, it is readily expected that the glucose content in the brain increases due to about a 50% increase in blood glucose content (Fig. 4B).

In all likelihood, both the amelioration of cerebral metabolism by a direct action on cerebral parenchyma and the indirect amelioration of cerebral metabolism through an increase of lowered blood flow play major roles in the beneficial effects of SAM on the incomplete ischemic brain.

Since ATP-dependent active Na+ transport is considered to be essential to cell volume regulation (34), in the present study, based on the results of energy metabolites, it is natural that edema was severe in the ischemic control group but was fairly suppressed in the SAM-treated group.

Further studies, especially studies on membrane lipids and cerebral blood flow, will be warranted to elucidate the detailed mechanism of the effects of SAM on ischemic brain.

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