Neurologia medico-chirurgica (1991.05) 31巻5号:251～256.

Relationship Between 22Na Distribution and Cerebral Blood Flow in Ischemic Gerbil Brain
(虚血性アレチネズミ脳における22Na分布と脳血流との関係)

Sako Kazuhiro, Yonemasu Yukichi

Neurol Med Chir (Tokyo) 31, 251~256, 1991

Relationship Between ^{22}Na Distribution and Cerebral Blood Flow in Ischemic Gerbil Brain

Kazuhiro SAKO and Yukichi YONEMASU

Department of Neurosurgery, Asahikawa Medical College, Asahikawa, Hokkaido

Abstract

Sodium transport in the early postischemic period was studied using Mongolian gerbils with right common carotid artery ligation. [^{22}Na]sodium chloride ([^{22}Na]NaCl) was infused immediately after, 10 minutes before, and 4 hours before carotid ligation, and the ^{22}Na distribution was measured in symptomatic animals by autoradiography 1 hour after ischemia. Regional cerebral blood flow was determined by [^{14}C]iodoantipyrine autoradiography. The specific gravity of the brain was measured in symptomatic gerbils 1 and 2 hours after carotid ligation by a gradient column. There was a low uptake of ^{22}Na in the ischemic core and a high uptake in the ischemic periphery when the tracer was given 10 minutes before or immediately after ischemia. In contrast, tracer given 4 hours before ischemia showed an increased radioactivity in both the ischemic core and periphery. It is suggested that increased sodium in the ischemic core is due to a decreased sodium clearance rate and increased sodium in the ischemic periphery is due to some active transport process.

Key words: cerebral edema, cerebral ischemia, blood-brain barrier, sodium transport, autoradiography, gerbil

Introduction

Klatzo's classification of cerebral edema[8] into vasogenic and cytotoxic edema has been widely accepted. A recent study of cerebral edema[4] using a modified specific gravity measurement method showed abnormal fluid accumulation in ischemic brain tissue within 5 minutes of arterial occlusion. No blood protein or tracer extravasation was observed in this stage.[7] The initial stages of ischemic edema can therefore be considered cytotoxic.

The pathogenic mechanism of ischemic brain edema in the early phase may be a sodium pump failure caused by a critical reduction in cerebral blood flow (CBF).[6,10] Sodium pump failure results in an influx of extracellular sodium into the intracellular compartment; the extracellular sodium is replaced by the sodium in the plasma.[6,20] The sodium concentration therefore increases in the brain tissue. The movement of water is thought to depend on the sodium concentration gradient. These experiments were designed to study the movement of sodium in the early postischemic period by [^{22}Na]sodium chloride ([^{22}Na]NaCl) autoradiography.

Received June 20, 1990; Accepted October 1, 1990

Materials and Methods

Male Mongolian gerbils weighing 60–80 gm were anesthetized with 1.5% halothane. The right common carotid artery was exposed through a ventral midline cervical incision, isolated from adjacent nerves and vessels, and doubly ligated with 4-0 silk sutures. After wound closure, the animals were allowed to recover from the anesthesia. Thirty minutes after ligation, the gerbils were observed for neurological symptoms and classified as symptomatic or asymptomatic according to the stroke index of Ohno *et al.*[16] Symptomatic animals only were used for the autoradiography and brain specific gravity measurement.

I. Measurement of regional CBF (rCBF)

rCBF was measured by the quantitative autoradiographic technique using [^{14}C]iodoantipyrine ([^{14}C]IAP) 1 hour after carotid ligation as described previously.[18] Ten μCi of [^{14}C]IAP was injected through the femoral vein for 1 minute at a constant infusion rate. Arterial blood was sampled every 6–8 seconds during tracer infusion to determine the [^{14}C]IAP concentration by liquid scintillation counting. At the end of tracer infusion, the gerbil was

decapitated, and the brain was rapidly removed and frozen in liquid freon-12 (−40°C). The frozen brain was sliced into 20 μm sections, which were mounted on cover glasses and dried for autoradiography. The brain sections were exposed for 1 week to single coated SB-5 x-ray films (Kodak, Rochester, N.Y., U.S.A.) in x-ray cassettes. The rCBF was calculated using the operational equation described by Sakurada *et al.*[19]

II. [^{22}Na]NaCl autoradiography

[^{22}Na]NaCl autoradiography was carried out in 28 animals. Thirty μCi of [^{22}Na]NaCl (553 mCi/mg sodium; New England Nuclear, Boston, Mass., U.S.A.) was injected through the femoral vein, immediately after (Group 1, n = 8), 10 minutes before (Group 2, n = 10), and 4 hours before carotid ligation (Group 3, n = 10). The tracer was infused under 1.5% halothane anesthesia.

The symptomatic animals, including three Group 1, three Group 2, and three Group 3, were reanesthetized with ether and decapitated 1 hour after carotid ligation. Their brains were quickly removed and frozen in liquid freon-12 (−40°C). [^{22}Na]NaCl autoradiography was carried out as described above.

III. [^{14}C]sucrose autoradiography

Immediately after carotid ligation, 60 μCi of [^{14}C]sucrose (420 mCi/mmol; American Radiolabeled Chemical Inc., St. Louis, Mo., U.S.A.) was injected through the femoral vein. The symptomatic gerbils (n = 3) were reanesthetized with ether and decapitated 1 hour after carotid ligation. Autoradiography was performed as described above.

IV. Measurement of brain specific gravity

The specific gravity of the symptomatic gerbil brains were measured 1 (n = 4) and 2 hours (n = 4) after carotid ligation using a gradient column containing bromobenzene and kerosene.[15] Standard solutions were prepared with distilled water and dried K_2SO_4 with specific gravities: 1.0324, 1.0365, 1.0406, 1.0447, 1.0489, and 1.0530. Duplicate measurements of the position in the column for each standard solution were used to prepare a standard curve. The animals were decapitated, and the brain was placed in a petri dish on ice during brain tissue sampling. Samples were obtained from the parietal cortex, hypothalamus, inferior colliculus, and cerebellum. The specific gravities of the tissue samples were determined from their positions 1 minute after insertion in the gradient column.

Results

I. rCBF

Representative [^{14}C]IAP autoradiograms are shown in Fig. 1. The rCBF measurements showed a severe decrease in the sensorimotor, parietal, and occipital cortices and a moderate reduction in the inferior colliculus and hypothalamus of the ischemic hemisphere (Table 1).

Table 1 rCBF value 1 hour after right common carotid artery ligation

Structure	Right	Left
Sensorimotor cortex	15 ± 3	129 ± 12
Parietal cortex	18 ± 4	110 ± 10
Occipital cortex	17 ± 8	126 ± 10
Cingulate cortex	79 ± 9	97 ± 12
Caudate putamen	38 ± 3	121 ± 11
Dorsolateral thalamus	58 ± 5	118 ± 10
Hypothalamus	22 ± 3	113 ± 9
Inferior colliculus	65 ± 8	134 ± 16
Cerebellar cortex	98 ± 9	102 ± 9

Values are means ± SEM (ml/100 gm/min) in three animals.

Fig. 1 Representative [^{14}C]IAP autoradiograms of a gerbil brain obtained 1 hour after carotid occlusion, showing a marked rCBF reduction in the ischemic hemisphere.

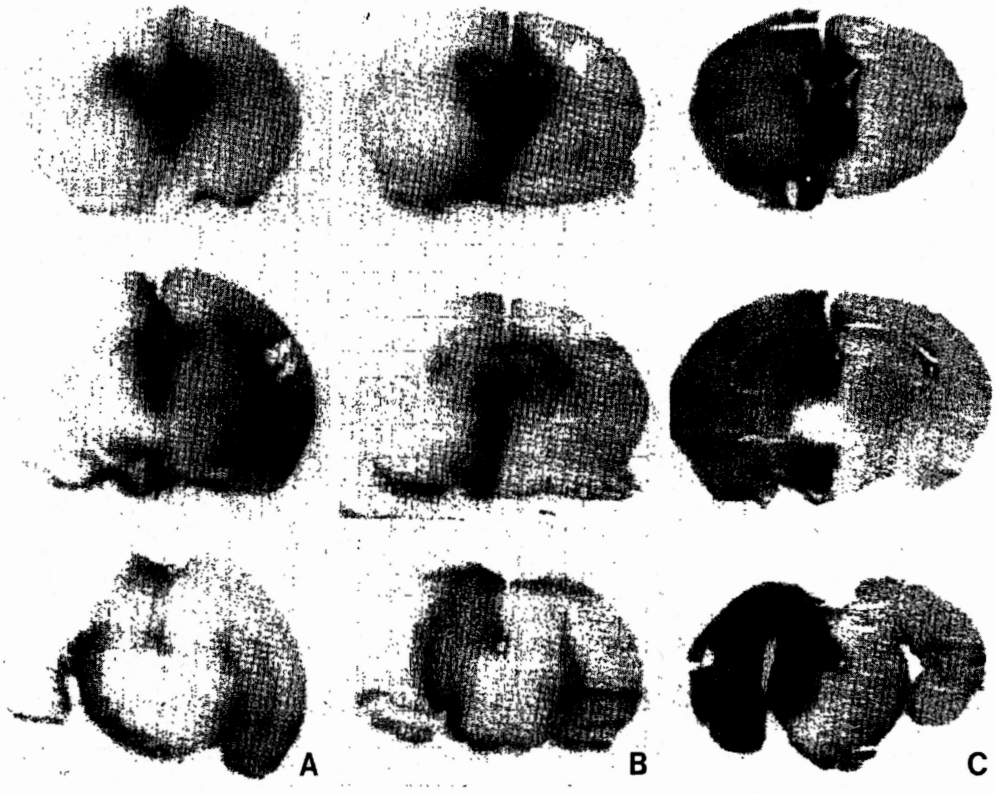

Fig. 2 Representative [^{22}Na]NaCl autoradiograms of a gerbil brain of Groups 1 (A), 2 (B), and 3 (C) in coronal sections at the level of inferior colliculus (*lower*), posterior hippocampus (*middle*), and caudate nucleus (*upper*).

II. [^{22}Na]NaCl autoradiography

Figure 2 shows representative [^{22}Na]NaCl autoradiograms, which demonstrate the heterogeneous distribution of the tracer. Concentrations were generally highest in the choroid plexus and in the regions adjacent to the cerebral ventricles and aqueduct of Sylvius.

The ratio of optical density in the ischemic to the contralateral side is given in Table 2. The symptomatic animals of Groups 1 and 2 demonstrated a low accumulation of ^{22}Na in the ischemic core and a high uptake in the periphery. In contrast, Group 3 animals demonstrated an increased radioactivity in both the ischemic core and periphery compared with the contralateral non-ischemic hemisphere.

Table 2 Ratio of optical density in ischemic to non-ischemic side 1 hour after right common carotid artery ligation

Structure	Group 1 (n = 3)	Group 2 (n = 3)	Group 3 (n = 3)
Parietal cortex	0.41 ± 0.08	0.46 ± 0.05	1.13 ± 0.02
Hypothalamus	1.26 ± 0.21	1.66 ± 0.35	1.67 ± 0.29
Inferior colliculus	1.22 ± 0.03	1.66 ± 0.07	1.31 ± 0.08
Cerebellum	0.98 ± 0.03	0.98 ± 0.01	1.00 ± 0.06

Values are means ± SD.

III. [^{14}C]sucrose autoradiography

[^{14}C]sucrose autoradiography showed a low accumulation in the ischemic area (Fig. 3). There was no increase in uptake in the ischemic periphery (hypothalamus, inferior colliculus).

IV. Specific gravity

Table 3 shows the specific gravity of the brain structures. One hour after ischemia, the specific gravity had decreased slightly in the ischemic core (parietal cortex) and moderately in the ischemic periphery (hypothalamus, inferior colliculus). Two hours after ischemia, the decrease in specific gravity of the ischemic core and periphery was statistically significant ($p < 0.05$).

Fig. 3 Representative [^{14}C]sucrose autoradiograms of a gerbil brain obtained 1 hour after carotid ligation, showing a low accumulation in the ischemic hemisphere.

Table 3 Brain specific gravity 1 and 2 hours after right common carotid artery ligation

Structure	1 hour after ischemia (n = 4)		2 hours after ischemia (n = 4)	
	Right	Left	Right	Left
Parietal cortex	1.0463 ± 0.0010	1.0476 ± 0.0008	1.0411 ± 0.0003[a,c]	1.0473 ± 0.0016
Hypothalamus	1.0437 ± 0.0017	1.0456 ± 0.0008	1.0409 ± 0.0033[a]	1.0457 ± 0.0014
Inferior colliculus	1.0438 ± 0.0030	1.0464 ± 0.0018	1.0400 ± 0.0024[b]	1.0467 ± 0.0024
Cerebellum	1.0482 ± 0.0003	1.0482 ± 0.0005	1.0476 ± 0.0008	1.0464 ± 0.0027

Values are means ± SD. Significantly different from left side at [a]$p < 0.05$ or [b]$p < 0.01$ or from 1 hour group at [c]$p < 0.05$ by Student's t-test.

Discussion

Unilateral carotid ligation in gerbil is a model of focal cerebral ischemia in which the CBF is severely reduced in the affected cerebral hemisphere. We used [^{14}C]IAP autoradiography to measure the quantitative rCBF, but our values slightly differed from those obtained by others using the same model and technique. Yoshimine *et al.*[24] reported higher rCBF values 30 minutes after unilateral common carotid artery occlusion, and Mies *et al.*[14] demonstrated lower values determined 2 hours after ischemia. Similar rCBF values were reported for the same model 1 hour after ischemia but using the [^{14}C]butanol diffusion technique.[11] These differences may result from the delay time between ischemic insult and rCBF measurement.

The degree of rCBF reduction somewhat varied among the animals. We chose the parietal cortex as the ischemic core and the hypothalamus and inferior colliculus as the ischemic periphery for the measurement of specific gravity, since these structures were easily identified and showed a relatively constant rCBF reduction. Low uptake of ^{22}Na in the ischemic core occurred when [^{22}Na]NaCl was given immediately after or 10 minutes before ischemia. Recently, Lo *et al.*[12] reported comparable results by direct counting of tissue radioactivity. They observed a 40% reduction in sodium permeability surface area product in the ischemic hemisphere compared with the non-ischemic hemisphere of gerbils with unilateral carotid ligation.

Many investigators have reported higher concentrations of sodium in the ischemic area than in the contralateral homologous area in the early postischemic period.[6,16,17,20,22] This has also been confirmed in gerbils 1 hour after unilateral carotid occlusion.[7] However, our data and the results of Lo *et al.*[12] showed low uptake of ^{22}Na in the ischemic core when [^{22}Na]NaCl was given immediately after or 10 minutes before ischemia. This indicates that the well-known increase in the net concentration of sodium in the ischemic area does not result from the enhanced sodium transport from plasma to brain. Our results may be explained by a reduction in the sodium clearance rate in the ischemic core, since [^{22}Na]NaCl autoradiograms indicate the turnover rate of sodium not the net concentration of sodium.

The net concentration of sodium in brain tissue is determined by the rate of influx from capillary to brain tissue and by the rate of efflux from brain tissue to plasma and cerebrospinal fluid space. Immediately after ischemia, sodium is transported from the extracellular to intracellular space.[6] Tracer infusion postischemia or 10 minutes preischemia resulted in most extracellular sodium being unlabeled, because sodium transport from capillary to brain tissue was very slow,[1,23] and therefore no increase in

radioactive sodium in the intracellular space was seen. If most extracellular sodium was labeled before ischemia, the net labeled sodium will increase after ischemia, because sodium will be replaced with labeled sodium from plasma. This was confirmed tracer infusion in gerbils 4 hours before ischemia demonstrating an increase in ^{22}Na uptake in the ischemic hemisphere.

In contrast, the ischemic periphery demonstrated increased ^{22}Na uptake with both pre- and postischemic tracer infusion. In other words, the increased sodium in the ischemic periphery came from the vascular compartment. The rCBF measurement showed a continuous supply of labeled sodium in these areas.

The next question was whether this increased sodium was by active or passive transport. Measurement of [^{14}C]sucrose, which was used as an internal standard for capillary permeability, revealed no increase in the ischemic periphery. Therefore, some active transport process is believed to be responsible.[3] Capillary Na,K-ATPase activity is thought to be such an active transport process.[2,13,21]

Thus, the mechanism responsible for the increased sodium in the ischemic core is probably different from that in the ischemic periphery. In the ischemic periphery, an increase in capillary Na,K-ATPase may be important,[2,13,21] while in the ischemic core, a decrease in the sodium clearance rate is probably responsible.[3,12] Sequential changes of tissue specific gravity in the ischemic core and periphery suggest that massive water movement may occur at the ischemic border zone. The water then moves to the ischemic core by bulk flow, driven perhaps by hydrostatic[9] or osmotic pressure.[5]

This study does not prove that the increased sodium in the ischemic core is derived from the ischemic periphery. This may be investigated by further study of the sequential changes of ^{22}Na distribution in the ischemic brain.

Acknowledgment

We thank Mr. H. Isobe for his excellent technical assistance.

References

1) Bakay L: Studies in sodium exchange. Experiments with plasma, cerebrospinal fluid, and normal, injured and embryonic brain tissue. *Neurology (Minneap)* 10: 564-571, 1960
2) Betz AL: Sodium transport in capillaries isolated from rat brain. *J Neurochem* 41: 1150-1157, 1983
3) Betz AL, Ennis SR, Schielke GP: Blood-brain barrier sodium transport limits development of brain edema during partial ischemia in gerbils. *Stroke* 20: 1253-1259, 1989
4) Fujimoto T, Walker JT Jr, Spatz M, Klatzo I: Pathophysiologic aspects of ischemic edema, *in* Pappius HM, Feindel W (*eds*): *Dynamics of Brain Edema.* Heidelberg, Springer, 1976, pp 171-180
5) Hatashita S, Hoff JT, Salamat SM: Ischemic brain edema and the osmotic gradient between blood and brain. *J Cereb Blood Flow Metab* 8: 552-559, 1988
6) Hossman K-A, Sasaki S, Zimmermann V: Cation activity in reversible ischemia of the cat brain. *Stroke* 8: 77-81, 1977
7) Ito U, Ohno K, Nakamura R, Suganuma F, Inaba Y: Brain edema during ischemia and after restoration of blood flow. *Stroke* 10: 542-547, 1979
8) Klatzo I: Neuropathological aspect of brain edema. *J Neuropath Exp Neurol* 26: 1-14, 1967
9) Kogure K, Busto R, Scheinberg P: The role of hydrostatic pressure in ischemic brain edema. *Ann Neurol* 9: 273-282, 1981
10) Leaf A: On the mechanism of fluid exchange of tissue in vitro. *Biochem J* 62: 241-248, 1956
11) Levy DE, Van Uitert RL: Postischemic cerebral blood flow studied with radioactive butanol, *in* Possonneau JV, Hawkins RA, Lust WD, Welsh FA (*eds*): *Cerebral Metabolism and Neural Function.* Baltimore, Williams & Wilkins, 1981, pp 186-195
12) Lo WD, Betz AL, Schielke GP, Hoff JT: Transport of sodium from blood to brain in ischemic brain edema. *Stroke* 18: 150-157, 1987
13) MacMillan V: Cerebral Na^+,K^+-ATPase activity during exposure to and recovery from acute ischemia. *J Cereb Blood Flow Metab* 2: 457-465, 1982
14) Mies G, Kloiber O, Drewes LR, Hossman K-A: Cerebral blood flow and regional potassium distribution during focal ischemia of gerbil brain. *Ann Neurol* 16: 232-237, 1984
15) Nelson SR, Mantz ML, Maxwell JA: Use of specific gravity in the measurement of cerebral edema. *J Appl Physiol* 30: 268-271, 1971
16) Ohno K, Ito U, Inaba Y: Regional cerebral blood flow and stroke index after left carotid artery ligation in the conscious gerbil. *Brain Res* 297: 151-157, 1984
17) Pappius HM: Evolution of edema in experimental cerebral infarction, *in* Prince TR, Nelson E (*eds*): *Cerebrovascular Disease.* New York, Raven, 1979, pp 131-139
18) Sako K, Kobatake K, Yamamoto YL, Diksic M: Correlation of local cerebral blood flow, glucose utilization, and tissue pH following a middle cerebral artery occlusion in the rat. *Stroke* 16: 828-834, 1985
19) Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L: Measurement of local cerebral blood flow with iodo(^{14}C)antipyrine. *Am J Physiol* 234: H59-H66, 1978
20) Schuier FJ, Hossmann K-A: Experimental brain in-

farcts in cats. II. Ischemic brain edema. *Stroke* 11: 593-601, 1980
21) Shigeno T, Asano T, Watanabe E, Johshita H, Takakura K: Dose capillary Na,K-ATPase plays a role in the development of ischemic brain edema, *in* Inaba Y, Klatzo I, Spatz M (*eds*): *Brain Edema, Proceedings of the Sixth International Symposium.* Berlin, Springer, 1985, pp 461-464
22) Siegel BA, Studer RK, Potchen EJ: Brain ^{22}Na uptake in experimental cerebral microembolism. *J Neurosurg* 38: 739-742, 1973
23) Smith QR, Rapoport ST: Cerebrovascular permeability coefficients to sodium, potassium, and chloride. *J Neurochem* 46: 1732-1742, 1986
24) Yoshimine T, Hayakawa T, Yamada K, Takemoto O, Kato A, Ushio Y, Mogami H: Regional cerebral ischemia in the gerbil: Measurement of regional cerebral blood flow by quantitative autoradiography. *J Cereb Blood Flow Metab* 6: 348-357, 1986

Address reprint requests to: K. Sako, M.D., Department of Neurosurgery, Asahikawa Medical College, 4-5 Nishikagura, Asahikawa, Hokkaido 078, Japan.