# Multi-focal Disturbances of the Postischemic Rat Brain by Measuring Blood Flow, Glucose Metabolism and Adenosine A1 Binding Activity

# III. 10. Multi-focal Disturbances of the Postischemic Rat Brain by Measuring Blood Flow, Glucose Metabolism and Adenosine $A_1$ Binding Activity

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## Introduction

It is well known that transient cerebral ischemia produces selectively neuronal damage in brain areas[1-3]. Neuronal degenerative processes occur especially in the hippocampus of the rat[4] and the Mongolian gerbil[5] induced by transient forebrain ischemia. Transient focal ischemia also induces neuronal degeneration in specific brain areas in the rat. Postischemic delayed neuronal damage has been reported in the ipsilateral thalamus and substantia nigra which lay outside ischemic areas of rat brain after middle cerebral artery (MCA) occlusion[6,7]. In those reports, the histological appearance of these remote areas was characterized by degeneration of most neurons with no necrotic changes of neuroglia and blood vessels. The mechanism of such a delayed phenomenon in the exo-focal remote areas is unclear, but it has been speculated that it might be caused by a transsynaptic process neuroanatomically associated with ischemic foci and that intracellular and transsynaptic signal transduction systems might play important roles in this mechanism[8-11].

Adenosine plays neuromodulatory roles in the central nervous system[12,13]. Especially, adenosine $A_1$ receptor inhibit adenylate cyclase activity[14] and mediate excitatory neuronal pathways by reducing neuronal activity[12]. The anatomical distribution of the adenosine receptor binding sites has been mapped in the rat brain by an autoradiographic method using a potent adenosine $A_1$ agonist [$^3$H]N$^6$-cyclohexyladenosine ([$^3$H]CHA)[15-17]. In the present study, we examined chronological changes of adenosine $A_1$ receptor binding sites of the rat brain, especially focusing on these changes in the exo-focal remote brain areas, after 90 min of MCA occlusion and after such occlusion followed by different periods of recirculation. We also investigated the mechanism involved by measuring regional cerebral blood flow (rCBF) and glucose metabolism.

## Materials and Methods

ISCHEMIA MODEL

Seventy-eight adult male Wistar rats of the SPF strain weighing 280-300 g were allowed free access to food and water before and after all procedures. Six rats were used for each experiment group. A detailed description of the surgical procedure has been previously reported[18]. In brief, after induction of anesthesia with a gas mixture of 70% $N_2O$ and 2% halothane (the balance being $O_2$), the right MCA was occluded with a silicone rubber cylinder attached to a nylon surgical thread introduced from the bifurcation of the internal carotid artery immediately after ligation of the ipsilateral common and external carotid arteries. The cylinder was made of 4-0 nylon surgical thread (Nitcho Kogyo Co., Ltd., Tokyo, Japan), 16 mm long. This cylinder was coated with silicone (Xantopren, Bayer Dental, Leverkusen, Germany) mixed with a hardener (Elastomer Activator, Bayer Dental) to increase the thickness of the distal 5 mm to 0.25-0.30 mm. After introducing the embolus, the internal carotid artery was ligated just distal to the point of insertion. The embolus extended from the bifurcation of the internal carotid artery to the proximal portion of the anterior cerebral artery (ACA). The origin of the right MCA and that of the right posterior communicating artery were occluded by the silicone rubber cylinder. In eighteen sham-operated control rats, the right internal and external carotid arteries were ligated. Surgery was performed within 15 min with no bleeding. Body temperature was kept at normal limits with a heating pad. Following surgery, anesthesia was discontinued and all rats exhibited neurologic deficits characterized by left hemiparesis with upper extremity dominancy and right Horner's syndrome. After 90 min of MCA occlusion, recirculation was achieved in 54 rats by pulling the thread out of the internal carotid artery under the same anesthetic conditions as during surgery. Once again, the rats were allowed free access to food and water. Although the ipsilateral common and external carotid arteries had been ligated, the ischemic area could be reperfused via the cerebral arterial circle (circle of Willis) through the contralateral carotid and basilar arteries, and by collateral circulation of cortical branches of the cerebral arteries.

### Adenosine $A_1$ receptor autoradiography

Adenosine $A_1$ receptor binding sites were measured using [$^3$H]cyclohexyladenosine ([$^3$H]CHA, spec. act. 34.4 Ci/mmol, Dupon NEN Products, Boston USA) according to the method of Onodera et al[19]. The rats were killed by decapitation 3 h, 6 h, 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after recirculation. After decapitation, the brains were quickly removed and frozen in powdered dry ice. Serial coronal sections 12 μm in thickness were cut on a cryostat (HM500, Zeiss, Germany) and thaw-mounted onto gelatin-coated slides. Sections were incubated with 5 nM [$^3$H]CHA and 2 units/ml adenosine deaminase (Boehringer-Mannheim, Mannheim, Germany) in 50 mM Tris-HC1 buffer (pH 7.4) at room temperature for 90 min. Following incubation, sections were washed in the buffer at 4 °C for 5 min and rapidly dried under a cold stream of air. Nonspecific binding was determined

using 10 μM L-phenylisopropyl-adenosine (Boehringer-Mannheim). Autoradiograms were prepared from the sections by exposing them to [$^3$H]sensitive hyperfilm (Amersham, Sweden AB, Solna, Sweden) with a tritium standard microscale (Amersham, International plc, Buckingham, UK) for 2 weeks in standard X-ray cassettes. The optical density of the brain regions was measured with a computed-assisted image analyzer (Zeiss, IBAS image analyzer system, Germany) without the examiner knowing the experimental protocol. The relationship between optical density and radioactivity was obtained with reference to [$^3$H]microscales co-exposed with the sections using a third-order polynominal function. The optical density of the brain regions measured in the present study was in the range where optical density and radioactivity of the [$^3$H]microscales showed a near linear relationship.

**Cerebral blood flow and glucose metabolism**

Regional CBF was measured in 6 sham-operated rats and in 6 rats 2 weeks of recirculation after 90 min of MCA occlusion. A tracheotomy was performed in 12 rats under the same anesthetic conditions and the rats were ventilated. Pancuronium bromide (0.6 mg/kg i.p.) was administered, and both femoral arteries and a femoral vein were cannulated. After surgical preparation, 2% halothane was discontinued and the rats were ventilated with 70% $N_2O$ and 30% $O_2$, allowing normoxia and normocapnia. CBF was measured by the [$^{14}$C]iodoantipyrine quantitative autoradiographic technique according to Sakurada et al. In brief, 20 μCi (0.6 ml) of 4-iodo-N-methyl-[$^{14}$C]iodoantipyrine (Amersham) was infused intravenously over 30 seconds. During the infusion, several 20-μl samples of arterial blood from the free-flowing femoral artery catheter were collected in sample tubes. The [$^{14}$C]iodoantipyrine concentration in the blood samples was determined by a liquid scintilation counter (Aloka) after allowing 24 hours for decolorization in a mixture with 1 ml tissue and gel solubilizer (Protosol) and 100 μℓ $H_2O_2$. The rats were decapitated approximately 30 seconds after the start of infusion. The brains were quickly removed and frozen in powdered dry ice. Each brain was sectioned (20 μm) in a cryostat (HM500, Zeiss, Germany) at -20 °C, and the sections were exposed to x-ray film (NMC-1, Kodak) with an autoradiographic carbon-14 standard microscale (Amersham) in x-ray cassettes for two weeks. Regional cerebral blood flow was calculated using a blood-brain coefficient of 0.8 and the equation derived by Sakurada et al.[20]

Regional cerebral glucose utilization (rCGU) was measured in other 6 rats after 90 min of ischemia followed by 2 weeks of recirculation and in 6 sham-operated rats. After the same surgical preparation as for the measurement of rCBF, samples of arterial blood were taken immediately prior to measurement of rCGU for determination of blood glucose level, $PaO_2$, $PaCO_2$, and pH. Regional CGU was measured by the 2-[$^{14}$C]deoxyglucose (Amersham) quantitative autoradiographic technique and rCGU was calculated using the equation of Sokoloff et al.[21] Cerebral [$^{14}$C] tissue concentrations of the autoradiograms were determined by means of a computerized microdensitometric system (Chromoscan, USA).

**Statistical analysis**

Values of adenosine $A_1$ binding sites were expressed as the means ±SD fmol/mg tissue using six animals. Data regarding the adenosine $A_1$ binding site, rCBF and rCGU in each structure of the brain were analyzed using Duncan's multiple range test with $p<0.05$ and $p<0.01$ considered to be statistically significant.

**Results**

ADNOSINE $A_1$ RECEPTOR BINDING ACTIVITY

The values of sham-operated control animals and chronological alteration of [$^3$H]CHA binding sites in each structure of rat brain after 90 min of MCA occlusion and after such occlusion followed by different periods of recirculation are summarized in Table 1. Representative autoradiograms of [$^3$H]CHA are shown in Figure 1. After 90-min ischemia followed by 1-day recirculation, significant decreases of the [$^3$H]CHA binding sites were first observed in the anterior neocortex (FrPaSS) ($p<0.05$) and the lateral segment of caudate putamen (CPu-L) ($p<0.01$), both of which were supplied by the occluded MCA. Therefore, [$^3$H]CHA binding sites of the ischemic side decreased to approximately 20% in the FrPaSS and to 35% in the CPu-L of each control value 4 weeks after the ischemic insult (Table 1). On the contrary, there was no alteration on day 1, but 3 days after recirculation, a significant reduction of [$^3$H]CHA binding sites was first detected in the ipsilateral thalamus ($p<0.05$), the amygdala ($p<0.05$), and the substantia nigra ($p<0.05$), which those areas were not directly affected by the original ischemic insult. Thereafter, the binding sites decreased progressively in the thalamus and the substantia nigra on the ischemic side (Figure 1). There were no significant changes of [$^3$H]CHA binding sites in the contralateral non-ischemic hemisphere.

**Cerebral blood flow and glucose metabolism**

In the measurement of rCBF and rCGU, there were no significant differences in physiological variables between the MCA occlusion and sham-operated groups. After 90 min of MCA occlusion followed by 2 weeks of recirculation, representative [$^{14}$C]iodoantipyrine and 2-[$^{14}$C]deoxyglucose autoradiograms of the thalamus and the substantia nigra are presented in Figure 2 and 3, respectively. There were no significant differences of rCBF in the exo-focal remote areas, the thalamus, the amygdala and the substantia nigra on the ischemic side compared with the each corresponding region of the contralateral hemisphere and sham-operated group (Table 2). However, rCGU in the ventral posterior nucleus of the thalamus on the ischemic side decreased inhomogeneously, to a variable extent, but was not significant compared with the corresponding region of the contralateral hemisphere and sham-operated group. On the other hand, CGU in the substantia nigra on the ischemic side

increased significantly ($p<0.01$) in comparison with the corresponding region of the contralateral hemisphere (Table 3).

## Discussion

It has been well known that adenosine and adenosine nucleotide play important roles in energy metabolism within cells as cyclic adenosine monophosphate and adenosine triphosphate (ATP). In experimental animals, a protective action of adenosine against ischemic neuronal damage has been demonstrated[22]. Furthermore, the adenosine receptor antagonist theophylline enhances ischemic neuronal damage in the gerbil hippocampus[23]. In general, adenosine is thought to prevent ischemic brain damage through several actions, such as enhanced resynthesis of ATP[24], inhibition of excitatory amino acid release[25], and reduction of free radical formation[26]. Therefore, adenosine may play an important role in the pathogenesis of ischemic brain damage[27].

The present study indicated that two different alterations of adenosine $A_1$ binding sites associated with the mechanisms of neuronal damage took place in the postischemic rat brain. First, in the frontoparietal cortex, somatosensory areas (FrPaSS), and the lateral segment of caudate putamen (CPu-L), adenosine $A_1$ binding activity decreased after 90 min of ischemia followed by 1 day of recirculation. In this model of ischemia, the FrPaSS and the CPu-L, which were supplied by the occluded MCA, were the regions most frequently damaged so-called ischemic foci[18]. We previously reported that more rapid changes of second messenger systems than adenosine $A_1$ binding activity were observed concurrent with abnormal calcium accumulation in the FrPaSS and the CPu-L on the ischemic side using the same ischemia model[10,11]. Regional CBF and glucose metabolism decreased persistently in those ischemic foci 2 weeks of recirculation after the ischemic insult. The reduction of adenosine $A_1$ binding activity in the FrPaSS and the CPu-L is explained by the direct damage to intracellular components including cell membrane following damage of second messenger systems and disruption of calcium homeostasis by ischemia-induced energy failure.

Second, in the exo-focal postischemic brain areas, the ipsilateral thalamus, the amygdala, the substantia nigra, significant decreases of adenosine $A_1$ binding activities were observed 3 days after the ischemia. These changes of adenosine $A_1$ binding activity observed in the thalamus and the substantia nigra on the ischemic side were concurrent with the abnormal calcium accumulation detected there in our previous study[6]. Moreover, both phenomena, i.e., the reduction of adenosine $A_1$ binding activity and abnormal calcium accumulation, in two remote areas on the ischemic side preceded the histologic findings of delayed neuronal damages. In contrast with the FrPaSS and the CPu-L, the ipsilateral thalamus and the substantia nigra were remote from these ischemic areas, and both areas had not been directly affected by the original ischemic insult[6]. Delayed neuronal damages in the exo-focal remote areas might be caused by a transsynaptic process associated with the ischemic foci[6].

However, regional glucose metabolism was quite different in two individual area, rCGU decreased in the thalamus and the amygdala, but increased in the substantia nigra, although there were no significant differences of rCBF in those exo-focal remote areas. We realize that the mechanisms of delayed neuronal damage in the exo-focal remote areas may be variable and are complicated in remote areas, i.e., the thalamus, the amygdala, and the substantia nigra on the ischemic side.

Iizuka et al. reported that delayed neuronal degeneration of the ipsilateral thalamus was observed after somatosensory cortical infarct of rats using Fink-Heimer silver staining method[28]. Reduction of adenosine $A_1$ binding activity in the thalamus on the ischemic side may be explained by retrograde neuronal degeneration due to thalamo-cortical fiber damage in ischemic cortical regions. Yamada et al. reported that basic fibroblast growth factor prevented neuronal degeneration of the thalamus after MCA occlusion in rats[29]. This indicates that trophic substances may play an important role in the mechanism of neuronal damage of the thalamus which might be caused by retrograde degeneration of the thalamo-cortical pathway after ischemic insult.

On the other hand, in the ipsilateral substantia nigra, it is not easy to explain the mechanism of the delayed reduction of adenosine $A_1$ receptor binding activity 3 days after the ischemic insult and increased glucose metabolism 2 weeks after the ischemia. We speculate as a possible hypothesis that the phenomenon in this area may be explained as being due to abnormal function of some neurotransmitters in the transsynaptic process associated with ischemic foci[11,30]. As for the substantia nigra, it consists of fibrous connections originating in the caudate putamen and projecting over the globus pallidus, forming the strio-nigral pathways[31]. The neuro-inhibitory transmitter GABA plays an important functional role in the strio-nigral pathway[32-34]. The increase of glucose metabolism in the substantia nigra on the ischemic side may be explained as being a result of altered neuronal function and hypermetabolism and is caused by diminished inhibitory output from the caudate putamen, which was affected by the precedent ischemic insult. We also speculate that the delayed neuronal degeneration in this area may be induced by disinhibitory overexcitation caused by diminished inhibitory regulation from the caudate putamen. Further detailed investigation is required in order to clarify the mechanisms of delayed neuronal degeneration in the exo-focal remote areas, which may be caused by neuronal network disturbances after ischemia.

Based on the present study, we conclude that postischemic alteration of adenosine $A_1$ binding activity was involved not only in the ischemic foci due to ischemia-induced energy failure, but also in the exo-focal remote areas prior to the histologic changes, which neuronal damage might be caused by the transsynaptic delayed degeneration associated with the ischemic foci. Furthermore, we suggest that multi-focal neuronal dysfunction in the postischemic brain areas may exacerbate the clinical symptoms of patients during the chronic stage of stroke.

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Table 1. Time-course of [$^3$H]CHA binding sites in each structure of the rat brain after 90 min of MCA occlusion followed by different periods of recirculation.

| Structure | Control | 90-min ischemia | 3 hours | 6 hours | 1 day |
|---|---|---|---|---|---|
| Ischemic side | | | | | |
| FrPaM | 233.8 ± 14.8 | 237.3 ± 15.4 | 233.2 ± 31.6 | 230.5 ± 30.7 | 225.9 ± 38.3 |
| FrPaSS | 236.9 ± 18.3 | 226.5 ± 19.4 | 241.6 ± 35.1 | 230.3 ± 24.1 | 192.8 ± 33.6* |
| CPu(L) | 214.4 ± 29.3 | 206.3 ± 13.5 | 226.8 ± 36.8 | 198.1 ± 18.0 | 138.6 ± 17.7** |
| CPu(M) | 214.9 ± 29.9 | 191.7 ± 19.1 | 220.7 ± 36.8 | 212.0 ± 37.4 | 236.6 ± 39.4 |
| Hippocampus | 311.1 ± 22.1 | 316.1 ± 37.8 | 349.7 ± 34.5 | 319.9 ± 24.1 | 356.7 ± 35.5 |
| Thalamus | 298.8 ± 36.8 | 290.6 ± 21.4 | 319.6 ± 39.0 | 281.1 ± 33.9 | 310.0 ± 38.0 |
| Amygdala | 121.5 ± 9.6 | 138.3 ± 9.0 | 141.2 ± 29.0 | 137.2 ± 14.9 | 129.3 ± 27.8 |
| Substantia nigra | 129.3 ± 15.4 | 113.1 ± 13.6 | 115.4 ± 27.3 | 114.8 ± 15.1 | 115.1 ± 20.1 |
| Pons | 93.4 ± 9.0 | 112.2 ± 12.2 | 102.4 ± 13.1 | 105.3 ± 13.1 | 100.1 ± 22.3 |
| Non-ischemic side | | | | | |
| FrPaM | 233.6 ± 21.7 | 236.5 ± 19.6 | 235.5 ± 24.9 | 232.9 ± 30.7 | 230.3 ± 47.6 |
| FrPaSS | 221.8 ± 24.6 | 225.3 ± 22.6 | 247.1 ± 22.3 | 228.5 ± 26.4 | 222.0 ± 38.4 |
| CPu(L) | 214.5 ± 34.4 | 220.6 ± 18.3 | 227.6 ± 18.4 | 221.0 ± 25.8 | 238.1 ± 22.8 |
| CPu(M) | 210.3 ± 35.9 | 205.2 ± 15.1 | 227.9 ± 12.2 | 217.5 ± 20.6 | 237.5 ± 24.9 |
| Hippocampus | 316.2 ± 39.3 | 319.1 ± 37.1 | 336.7 ± 33.6 | 336.4 ± 26.7 | 345.1 ± 36.6 |
| Thalamus | 295.6 ± 36.9 | 304.2 ± 33.1 | 312.1 ± 30.3 | 278.1 ± 27.4 | 285.9 ± 27.4 |
| Amygdala | 124.4 ± 20.6 | 133.7 ± 21.5 | 115.7 ± 19.1 | 144.7 ± 22.3 | 134.9 ± 25.8 |
| Substantia nigra | 117.0 ± 21.2 | 118.3 ± 6.7 | 120.9 ± 20.1 | 113.4 ± 17.7 | 120.4 ± 33.9 |
| Pons | 99.6 ± 8.4 | 111.1 ± 10.3 | 106.7 ± 12.5 | 103.5 ± 14.8 | 103.4 ± 17.4 |

Values are given in Mean ± S.D. fmol/mg tissue using six animals.
FrPaM: frontoparietal cortex, motor area, supplied by anterior cerebral artery;
FrPaSS: frontoparietal cortex, somatosensory area, supplied by middle cerebral artery;
CPu(L): lateral segment of caudate putamen; CPu(M): medial segment of caudate putamen;
*p<0.05; **p<0.01, significant difference from control values using Duncan's multiple range test.

Table 1. Time-course of [$^3$H]CHA binding sites in each structure of the rat brain after 90 min of MCA occlusion followed by different periods of recirculation (continued).

| Structure | 3 days | 1 week | 2 weeks | 4 weeks |
|---|---|---|---|---|
| Ischemic side | | | | |
| FrPaM | 236.4 ± 36.8 | 233.8 ± 22.6 | 240.1 ± 23.8 | 238.7 ± 33.4 |
| FrPaSS | 74.2 ± 6.9** | 82.9 ± 24.6** | 55.1 ± 11.3** | 52.2 ± 7.0** |
| CPu(L) | 97.4 ± 24.6** | 102.1 ± 19.7** | 81.5 ± 12.7** | 71.6 ± 8.4** |
| CPu(M) | 233.9 ± 20.6 | 234.2 ± 38.3 | 233.2 ± 20.5 | 231.3 ± 22.1 |
| Hippocampus | 353.2 ± 27.3 | 322.8 ± 18.9 | 368.5 ± 22.3 | 362.2 ± 36.1 |
| Thalamus | 225.1 ± 17.9** | 179.0 ± 28.4** | 163.6 ± 29.0** | 166.5 ± 12.5** |
| Amygdala | 105.5 ± 22.3* | 82.6 ± 20.6** | 79.6 ± 28.1** | 58.9 ± 15.6** |
| Substantia nigra | 99.1 ± 15.4* | 87.1 ± 11.9** | 75.1 ± 12.2** | 60.4 ± 14.4** |
| Pons | 101.1 ± 24.9 | 109.2 ± 11.0 | 106.4 ± 7.3 | 118.6 ± 17.9 |
| Non-ischemic side | | | | |
| FrPaM | 232.2 ± 27.6 | 236.5 ± 22.9 | 254.6 ± 33.3 | 250.6 ± 29.3 |
| FrPaSS | 261.1 ± 35.4 | 254.9 ± 30.9 | 259.1 ± 37.4 | 231.8 ± 35.1 |
| CPu(L) | 249.7 ± 23.5 | 250.0 ± 21.0 | 250.7 ± 21.9 | 258.6 ± 30.7 |
| CPu(M) | 228.0 ± 29.0 | 236.1 ± 26.7 | 239.6 ± 25.2 | 232.1 ± 27.6 |
| Hippocampus | 350.0 ± 27.7 | 347.7 ± 22.3 | 348.2 ± 23.8 | 332.8 ± 20.3 |
| Thalamus | 285.6 ± 26.8 | 309.1 ± 25.1 | 304.0 ± 34.8 | 302.5 ± 37.1 |
| Amygdala | 154.7 ± 28.1 | 152.5 ± 16.1 | 150.0 ± 20.0 | 150.2 ± 14.8 |
| Substantia nigra | 115.5 ± 11.6 | 114.3 ± 17.9 | 130.4 ± 10.7 | 130.2 ± 11.2 |
| Pons | 100.4 ± 12.5 | 104.3 ± 11.0 | 113.4 ± 10.6 | 118.1 ± 13.1 |

Values are given in Mean ± S.D. fmol/mg tissue using six animals.
FrPaM: frontoparietal cortex, motor area, supplied by anterior cerebral artery;
FrPaSS: frontoparietal cortex, somatosensory area, supplied by middle cerebral artery;
CPu(L): lateral segment of caudate putamen; CPu(M): medial segment of caudate putamen;
*p<0.05; **p<0.01, significant difference from control values using Duncan's multiple range test.

Table 2. Regional cerebral blood flow of the sham-operated control rats and measured after 90-min ischemia followed by 2-week recirculation.

| Structure | sham-operated control group | 90-min ischemia followed by 2-week recirculation |
|---|---|---|
| Ischemic side | | |
| FrPaM | 1.39 ± 0.09 | 1.36 ± 0.14 |
| FrPaSS | 1.70 ± 0.18 | 0.55 ± 0.08** |
| CPu(L) | 1.65 ± 0.15 | 0.99 ± 0.17** |
| CPu(M) | 1.63 ± 0.12 | 1.62 ± 0.10 |
| Hippocampus | 1.01 ± 0.10 | 0.90 ± 0.09 |
| Thalamus (VPN) | 1.62 ± 0.12 | 1.47 ± 0.14 |
| Amygdala | 1.19 ± 0.17 | 0.51 ± 0.08** |
| Substantia nigra | 1.05 ± 0.08 | 0.98 ± 0.16 |
| Pons | 1.42 ± 0.12 | 1.34 ± 0.12 |
| Non-ischemic side | | |
| FrPaM | 1.41 ± 0.09 | 1.42 ± 0.09 |
| FrPaSS | 1.68 ± 0.15 | 1.63 ± 0.23 |
| CPu(L) | 1.62 ± 0.12 | 1.78 ± 0.05 |
| CPu(M) | 1.60 ± 0.13 | 1.77 ± 0.07 |
| Hippocampus | 1.05 ± 0.09 | 0.94 ± 0.12 |
| Thalamus (VPN) | 1.59 ± 0.11 | 1.60 ± 0.11 |
| Amygdala | 1.17 ± 0.15 | 1.33 ± 0.19 |
| Substantia nigra | 1.07 ± 0.10 | 0.96 ± 0.08 |
| Pons | 1.40 ± 0.13 | 1.39 ± 0.08 |

Values are given in mean ± SD ml/g/min using six animals. FrPaM, frontoparietal cortex, motor area, supplied by anterior cerebral artery; FrPaSS, frontoparietal cortex, somatosensory area, suuplied by middle cerebral artery; CPu(L), lateral segment of caudate putamen; CPu(M), medial segment of caudate putamen; Thalamus (VPN), ventral posterior nucleus of thalamus. **$p<0.01$, significant difference from control values using Duncan's multiple range test.

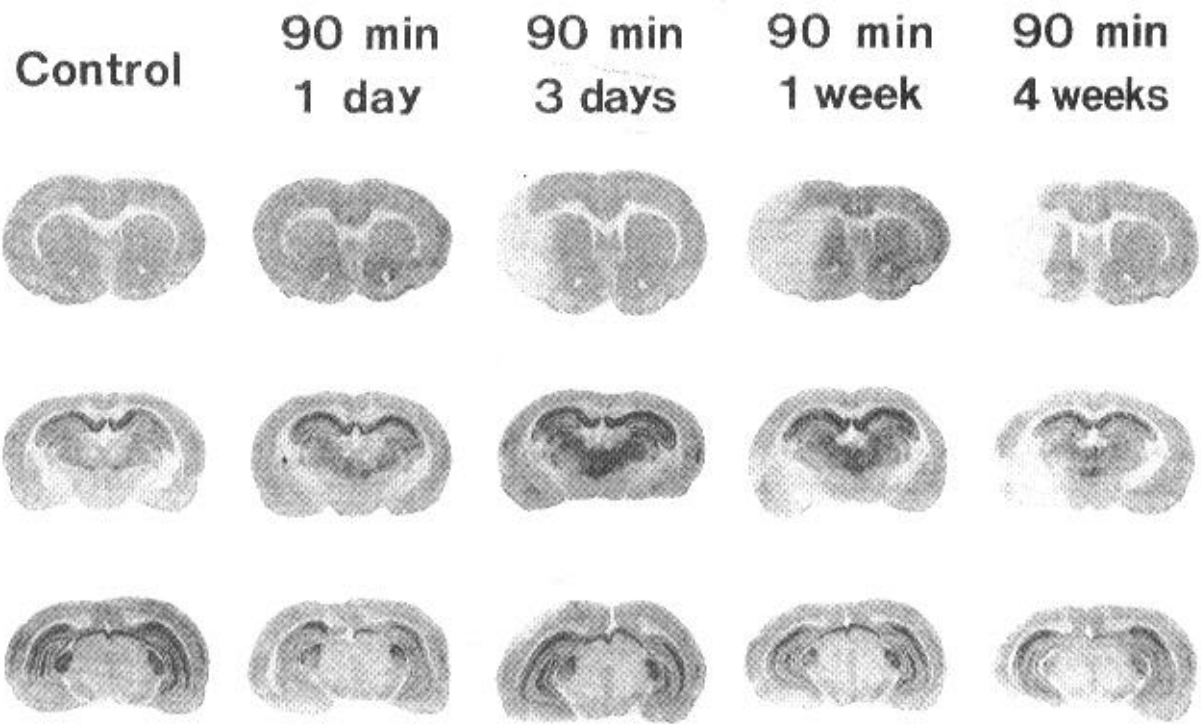


Fig. 1. [$^3$H]CHA autoradiograms of the brains of sham-operated control rats and those obtained after 90 min of MCA occlusion followed by 1-day, 3-day, 1-week, and 4-week recirculation. Representative autoradiograms show coronal sections at the level of the caudate putamen (top), the thalamus (middle), and the substantia nigra (bottom).

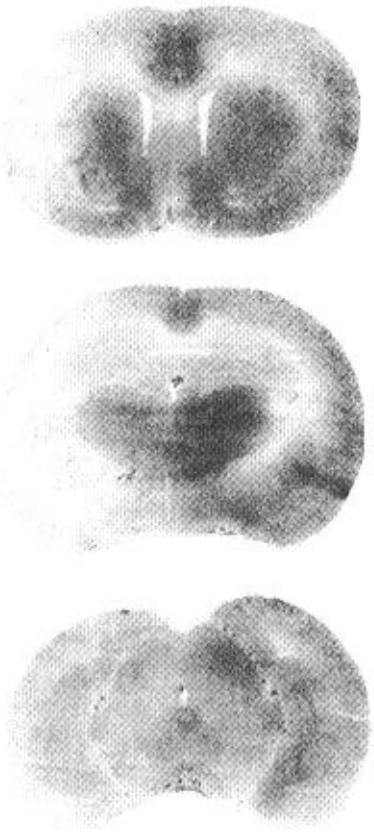

Fig. 2. [$^{14}$C]iodoantipyrine autoradiograms of the rat brain after 90 min of MCA occlusion followed by 2 weeks of recirculation. Representative autoradiograms show coronal sections at the level of the caudate putamen (top), the thalamus (middle), and the substantia nigra (bottom).

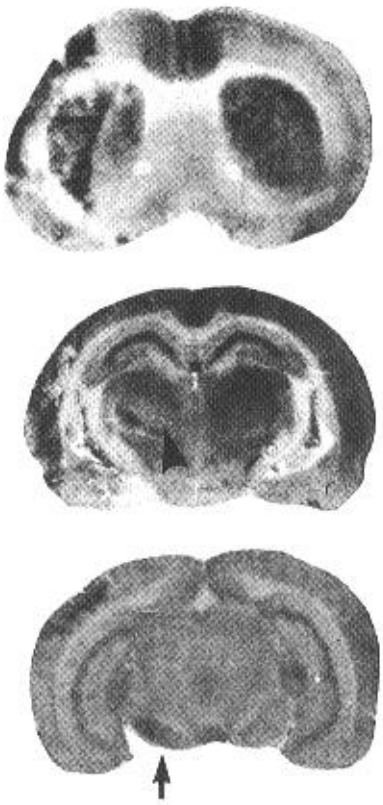

Fig. 3. 2-[$^{14}$C]Deoxyglucose autoradiograms of rat brain after 90 min of MCA occlusion followed by 2 weeks of recirculation. Regional cerebral glucose utilization (rCGU) decreases slightly and inhomogeneously in the ventral posterior nucleus of the thalamus of the ischemic side (arrowhead), whereas an increase in rCGU is seen in the ipsilateral substantia nigra (arrow).