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Introduction

Transient cerebral ischemia results in a selective pattern of neuronal degeneration within the central nervous system in both humans and experimental animals. We have previously reported that ischemic neuronal damage after transient focal ischemia was observed not only in the ischemic foci which supplied by the occluded artery, but also in the exo-focal remote areas which had not been affected by the original ischemic insult [1]. The mechanism of delayed neuronal degeneration 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 [2-4]. Recent studies suggest that postischemic alterations of second messenger systems play a key role in the pathogenesis of neuronal damage after ischemia [5-7].

Rolipram ((+)-4-(3-cyclopentyloxy-4-methoxy) phenyl-2-pyrrolidone) is a clinically effective antidepressant with selective cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE) inhibiting properties [8] and inhibits a Ca^{2+}/calmodulin independent cAMP selective PDE isoenzyme leading to increase cAMP levels of the brain [9-13]. Specific binding sites for [^{3}H] rolipram have been described in rat and human brain [14]. Regional variations in the density of these binding sites of the brain can be visualized and quantified by autoradiographic method [15]. The rolipram binding protein has recently been separated from other PDE isoenzymes and identified as a type of PDE with an affinity for cAMP [12]. It is thought that autoradiographic analysis of these binding sites can delineate neuronal damage of the postischemic rat brain as a marker of second messenger system. In the present study, we examined chronological changes of cyclic adenosine monophosphate PDE by measuring rolipram binding sites of the rat brain after 90 min of middle cerebral artery (MCA) occlusion and after such occlusion followed by different periods of recirculation in order to clarify the mechanisms of postischemic neuronal damage.

Materials and Methods

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 in each experiment. A detailed description of the surgical procedure has been previously reported [16]. 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. In six sham-operated control rats, the right internal and external carotid arteries were ligated. After 90 min of MCA occlusion, the six rats were decapitated with no recirculation, and in other rats, recirculation was achieved by pulling the thread out of the internal carotid artery under the same anesthetic conditions as during surgery.

The localization and the chronological changes of cyclic adenosine monophosphate phosphodiesterase were measured using radiolabeled [^{3}H] rolipram (New England Nuclear, spec. act. 31 Ci/mmol) by the method of Kaulen et al [15]. The rats were killed by decapitation after 90 min of ischemia and after such ischemia followed by 3 h, 6 h, 1 day, 3 days, 1 week, 2 weeks, and 4 weeks of recirculation. After decapitation, the brains were quickly removed and frozen in powdered dry ice and stored at -80 °C until assay. Serial coronal sections 12 μm in thickness were cut on a cryostat and thaw-mounted onto gelatin-coated slides. Brain sections were incubated for 60 min at 4°C in 150 mM phosphate buffer (pH 7.4) containing 2 mM $MgCl_2$, 100 μM dithiothreitol, and 5 nM [^{3}H] rolipram. Following incubation, sections were washed twice for 30 s each at 4 °C in the same buffer, rinsed in distilled water, and dried rapidly under a cold stream of air. Non-specific binding was calculated in the presence of unlabeled 1 μM rolipram (Sigma). Autoradiograms were prepared from the sections by exposing them to [^{3}H] sensitive hyperfilm (Amersham) with tritium standard microscale (Amersham) for 6 weeks in standard X-ray cassettes.

Cerebral [^{3}H] tissue concentrations of the autoradiograms were determined by means of a computerized microdensitometric system. Data regarding the rolipram binding sites in each structure of the brain were analyzed using a t-test with $p < 0.05$ and $p < 0.01$ considered to be statistically significant.

Results and Discussion

Chronological alteration of [^{3}H] rolipram autoradiograms of the brains of sham-operated control rats and those obtained after 90 min of ischemia followed by different periods of recirculation is shown in Fig. 1. In the present study, three different alterations of the second messenger system were observed in each area of the postischemic rat brain.

First, in the ischemic foci, the ipsilateral anterior neocortex (FrPaSS) and the lateral part of the caudate putamen (CPu-L), [^{3}H] rolipram binding sites were rapidly decreased after 90-min ischemia with no recirculation; thereafter, these sites were progressively reduced in both areas (Table l). Such a rapid change of [^{3}H] rolipram binding sites in the ischemic foci occurred concurrently with the alterations of other second messenger systems by measuring forskolin and inositol l,4,5-trisphosphate binding sites using the same ischemia model [6,7]. These rapid changes in the ischemic foci are explained by direct damage to intracellular components by ischemia induced energy failure.

Second, a significant reduction of rolipram binding sites was observed in the amygdala on the ischemic side 6 hours after the ischemia; thereafter, these sites were progressively reduced (Table l). The mechanism of this change observed in the amygdala is not easily explained. It is known that the amygdala is one of the most vulnerable areas against an ischemic insult [17] and that the amygdala consisting of limbic systems has transsynaptic fiber connections with the ipsilateral ischemic foci, the FrPaSS and the CPu-L. Moreover, the high affinity of the rolipram binding sites was reported in the amygdala of the rat brain consisting of the limbic systems which were enriched in rolipram sensitive PDE isoenzyme [15]. The postischemic early change of the binding site in the amygdala may reflect high susceptibility to ischemic iniury and easy detection due to the initial high binding activity. The enrichment of rolipram binding sites in the limbic systems involved in the regulation of emotion and cognition can be regarded as the neuroanatomical correlation of pharmacological properties of rolipram as an antidepressant.

Third, in the ipsilateral thalamus and the substantia nigra which lay outside the original ischemic areas, [^{3}H] rolipram binding sites were not changed with the control values 1 day after the ischemic insult, and thereafter a significant reduction of these binding sites was first observed 3 days after the ischemia (Fig. 1, Table 1). This delayed change of second messenger system observed in the thalamus and the substantia nigra was quite similarly developed as abnormal calcium accumulation was detected using the same ischemia model [1]. The alteration of [^{3}H] rolipram binding sites in the substantia nigra was minimal because of their initial low binding activity [15]. There were no significant changes of rolipram binding sites in the contralateral non-ischemic hemisphere during and after the ischemia.

Since rolipram is considered to exert its antidepressant properties by inhibiting a selective cAMP PDE isoenzyme modifying cAMP levels of the brain [18-20], its biochemical actions may have a close relationship with signal transduction system related to cAMP as a second messenger. It may be congruent to discuss the relationship between the anatomical distribution of rolipram binding sites and those areas of the brain that employ cAMP. In our previous study, the histological appearance of the thalamus and the substantia nigra, which had not been directly affected by original ischemic insult, was characterized by selective neuronal degeneration of most neurons with no necrotic changes of neuroglia and blood vessels [1]. The present results indicate that alteration of second messenger system may be

involved not only in the ischemic foci, but also in neuronal degeneration of the exo-focal postischemic brain areas and that alteration of intracellular signal transduction may precede the degenerative neuronal damage in the exo-focal remote areas. We suggest that this delayed multi-focal brain damage including the limbic systems may exacerbate clinical symptoms in the chronic stage of cerebral vascular disease.

References

1) Nagasawa H. and Kogure K., Brain Res. **524** (1990) 196.
2) Kogure K., Tanaka J. and Araki T., Neurochem. Pathol. **9** (1988) 145.
3) Raichle M. E., Ann Neurol. **13** (1983) 2.
4) Siesjö B. K., J. Cereb. Blood Flow Metab. **1** (1981) 155.
5) Jorgensen M. B., Deckert J. and Wright D. C., Neurosci. Lett.**103** (1989) 219.
6) Nagasawa H. and Kogure K., Brain Res. **563** (1991) 7.
7) Nagasawa H. and Kogure K., Neurosci. Lett. **133** (1991) 129.
8) Zeller E. et al., Pharmacopsychiatry **17** (1984) 188.
9) Davis C. W., Biochem. Biophys. Acta. **797** (1985) 354.
10) Ilien B., Ruckstuhl M. and Landry Y., J. Pharmacol. **13** (1982) 307.
11) Schneider H. H., Biochem. Pharmacol. **33** (1984) 1690.
12) Schneider H. H., Pahlke G. and Schmiechen R., Sensitivity of a cAMP PDE to rolipram in different organs. In L.M.G. Heilmeyer (Ed.), Signal Transduction and Protein Phosphorylation, NATO ASI, Series A, Vol. 135, Plenum Press, New York and London,1987, pp. 81-85.
13) Schwabe U. et al., Mol. Pharmacol. **12** (1976) 900.
14) Schneider H. H. et al., Eur. J. Pharmacol. **127** (1986) 105.
15) Kaulen P. et al., Brain Res. **503** (1989) 229.
16) Nagasawa H. and Kogure K., Stroke **20** (1989) 1037.
17) Pulsinelly W. A., Prog. Brain Res. **63** (1985) 29.
18) Wachtel H., Neuropharmacology **22** (1983) 267.
19) Wachtel H. and Schneider H. H., Neuropsychopharmacology **25** (1986) 1119 .
20) Wachtel H. et al., Neurosci. Lett. **76** (1987) 191.

Table 1. (^{3}H)rolipram binding 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	94.2 ± 5.7	90.4 ± 6.1	93.9 ± 5.1	93.2 ± 5.1	97.7 ± 5.1
FrPaSS	92.9 ± 5.7	76.0 ± 4.2**	69.0 ± 2.2**	64.2 ± 4.2**	61.6 ± 2.6**
CPu(L)	84.0 ± 3.5	74.4 ± 3.8**	69.9 ± 2.2**	66.1 ± 5.7**	61.6 ± 1.9**
CPu(M)	82.4 ± 4.2	81.4 ± 2.2	87.5 ± 5.1	86.2 ± 7.3	87.2 ± 3.2
Hippocampus	99.3 ± 1.9	99.6 ± 7.7	96.4 ± 4.2	99.0 ± 9.9	100.9 ± 8.6
Thalamus	99.9 ± 4.2	93.2 ± 1.9	95.8 ± 6.7	95.5 ± 4.2	91.6 ± 8.3
Amygdala	69.9 ± 2.9	69.3 ± 3.8	69.9 ± 2.6	61.6 ± 3.8*	58.4 ± 2.9*
Substantia nigra	56.0 ± 4.2	56.2 ± 4.5	54.9 ± 2.9	55.2 ± 1.3	52.0 ± 1.0
Pons	50.1 ± 2.2	51.4 ± 3.8	52.0 ± 2.6	54.0 ± 4.5	50.2 ± 3.5
Non-ischemic side					
FrPaM	93.9 ± 1.9	95.6 ± 4.5	91.0 ± 3.8	95.5 ± 1.6	97.4 ± 3.8
FrPaSS	89.7 ± 9.9	92.0 ± 4.5	90.0 ± 5.7	91.6 ± 5.7	89.7 ± 3.2
CPu(L)	81.4 ± 3.2	87.2 ± 6.7	87.8 ± 3.2	91.0 ± 3.8	91.0 ± 2.6
CPu(M)	81.4 ± 5.1	85.3 ± 4.2	87.5 ± 4.2	86.8 ± 4.2	87.8 ± 2.6
Hippocampus	99.9 ± 2.6	92.6 ± 5.4	93.9 ± 6.1	97.7 ± 9.6	105.0 ± 8.3
Thalamus	98.7 ± 4.8	89.4 ± 7.7	92.6 ± 8.3	92.3 ± 4.5	92.0 ± 9.9
Amygdala	75.3 ± 4.2	73.1 ± 4.2	72.5 ± 3.2	68.6 ± 3.8	74.4 ± 3.8
Substantia nigra	56.8 ± 3.8	56.5 ± 5.7	54.9 ± 4.2	55.9 ± 3.2	54.6 ± 1.6
Pons	53.0 ± 2.6	50.4 ± 4.2	50.1 ± 2.9	49.2 ± 3.2	49.2 ± 1.3

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 a t-test.

Table 1. (^{3}H)rolipram binding 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	94.8 ± 4.5	94.8 ± 4.2	93.2 ± 9.9	91.6 ± 3.5
FrPaSS	46.6 ± 2.2**	43.7 ± 1.9**	37.7 ± 2.6**	33.2 ± 1.6**
CPu(L)	56.8 ± 2.9**	54.0 ± 3.2**	46.9 ± 4.2**	41.5 ± 3.2**
CPu(M)	85.7 ± 2.6	83.0 ± 4.8	89.4 ± 9.9	86.0 ± 4.5
Hippocampus	100.6 ± 9.9	98.7 ± 7.0	96.4 ± 6.7	93.9 ± 4.8
Thalamus	56.8 ± 7.3**	57.2 ± 5.1**	57.4 ± 4.5**	52.0 ± 2.2**
Amygdala	45.7 ± 2.6**	50.4 ± 3.2**	46.9 ± 4.8**	44.7 ± 7.0**
Substantia nigra	48.5 ± 2.2*	49.8 ± 1.6*	48.4 ± 3.2*	48.4 ± 3.3*
Pons	49.8 ± 3.5	47.6 ± 4.5	47.3 ± 4.5	47.9 ± 4.5
Non-ischemic side				
FrPaM	96.4 ± 4.2	98.0 ± 7.0	96.1 ± 4.8	96.4 ± 5.7
FrPaSS	92.3 ± 4.2	90.4 ± 2.6	88.8 ± 4.8	87.5 ± 2.9
CPu(L)	87.2 ± 2.2	93.9 ± 4.2	92.0 ± 7.0	88.1 ± 2.9
CPu(M)	83.7 ± 4.2	91.3 ± 5.4	90.0 ± 9.3	87.5 ± 3.2
Hippocampus	102.5 ± 7.3	98.7 ± 9.9	97.4 ± 8.0	95.2 ± 6.1
Thalamus	88.4 ± 9.9	88.8 ± 6.7	89.1 ± 6.1	92.6 ± 6.1
Amygdala	74.4 ± 5.4	77.0 ± 6.7	79.8 ± 5.7	82.1 ± 7.3
Substantia nigra	56.2 ± 3.5	53.6 ± 3.8	54.9 ± 4.5	53.3 ± 3.2
Pons	48.8 ± 1.9	48.9 ± 4.8	50.1 ± 2.9	50.1 ± 5.1

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 a t-test.

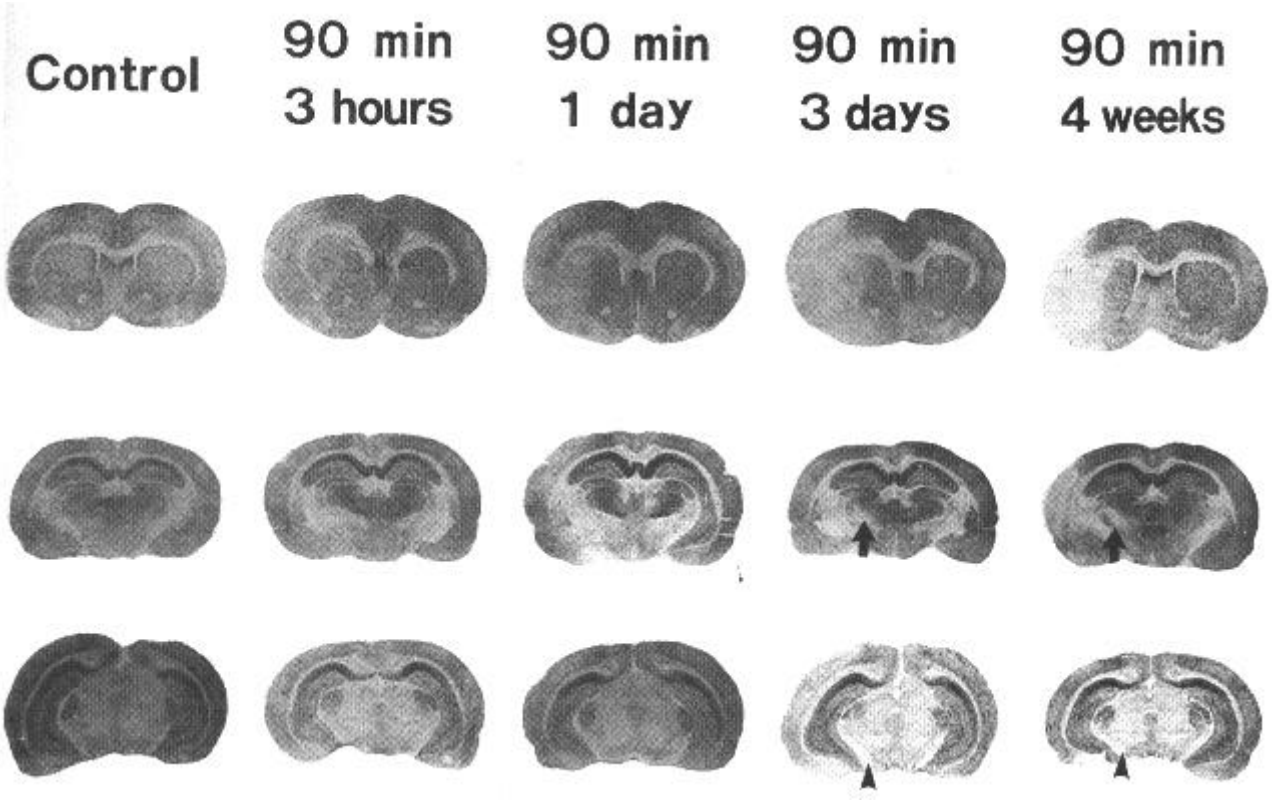

Fig. 1. [^{3}H] rolipram autoradiograms of the brains of sham-operated control rats and those obtained after 90 min of MCA occlusion followed by 3-hour, 1-day, 3-day, and 4-week recirculation. Representative autoradiograms show coronal sections at the level of the caudate putamen (top), the thalamus (middle), and the substantia nigra (bottom). Three days after the ischemia, the binding sites were first detected in the thalamus (arrow) and the substantia nigra (arrowhead) on the ischemic side and these findings continued up to 4-week recirculation period.