Sustained Changes in Acetylcholine and Amino Acid Contents of Brain Regions Following Microsphere Embolism in Rats

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ABSTRACT--The present study was undertaken to explore changes in neurotransmitters and neuromodulators of brain regions impaired by microsphere embolism-induced, sustained ischemia. Nine hundred microspheres (48 μm) were injected into the right internal carotid artery of rats, and the time course of changes in the triphenyltetrazolium chloride (TTC)-stained areas of their brain slices and acetylcholine and amino acid contents in the cerebral cortex, striatum and hippocampus of both hemispheres were determined. The TTC-unstained area, a measure of infarction, was developed in the right hemisphere by the 3rd day after the embolism, which was similar to that on the 28th day. A marked decline in acetylcholine content of these three regions of the right hemisphere was detected throughout the experiment (28 days). The glutamate, aspartate, GABA, and taurine levels were markedly decreased following microsphere-embolism. Most of these decreases were significantly attenuated during the first 5 days following the embolism, and they then partially recovered with time after the operation. Minor metabolic changes were observed in the left hemisphere. The results suggest that microsphere-embolism induces cerebral infarction and/or sustained damage to acetylcholine and neurotransmitter amino acid synthesis and/or catabolism of the brain regions. This model may provide information concerning the pathophysiological alterations in long-term cerebral ischemia and infarction.

Keywords: Acetylcholine, Amino acid, Choline, Ischemia (brain), Microsphere-embolism

Cerebral embolism induced by blood clots (1), microspheres (2) or silicon rubber (3) has been shown to result in cerebral oligemia or ischemia and eventually lead to functional, biochemical and morphological damage to the brain in small experimental animals. Embolism with microspheres, which is considered irreversible due to their physical properties, has been shown to induce widespread, focal ischemia, followed by cerebral infarction (4-7). Most of these studies, however, concerned the pathophysiology of short-term ischemia and thus do not provide information concerning long-term cerebral ischemia. We considered that induction of sustained or irreversible damage in the brain of experimental animals may provide useful information concerning the development of, protection against, and therapeutics for ischemic brain diseases such as cerebral infarction, stroke and infarct-induced dementia. In a previous study (8), we examined brain energy metabolism following microsphere-induced cerebral embolism with the same aim as above on the basis of changes in biochemical parameters including tissue high-energy phosphate, lactate, glucose and glycogen contents of the brain regions. In the present study, we attempted to explore the pathophysiological changes in cholinergic neurotransmitters and putative neurotransmitter and neuromodulator amino acids of the brain following microsphere-embolism. Furthermore, we examined changes in these metabolites in three regions of the brain, the cerebral cortex, striatum and hippocampus, since these regions are recognized to be extremely sensitive to oxygen-deficiency (9-11).

MATERIALS AND METHODS

Surgery for inducing cerebral embolism

Male Wistar rats weighing 180 to 220 g (Charles River Japan, Inc., Atsugi) were used in the present study. The

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animals were maintained at 23±1℃ with a constant humidity of 55±5% with a 12 hr-cycle of light and dark, and they were given free access to food and tap water; The housing conditions and treatment of the animals were in accord with the Guideline of Experimental Animal Care issued by the Japanese Prime Minister's Office. Surgery for microsphere-induced cerebral embolism was performed by the previously described method (8). Briefly, 132 rats were anesthetized with 30 mg/kg of sodium pentobarbital, i.p. and fixed in the supine position on an operation plate. After the right external carotid and the right pterygopalatine arteries were ligated with strings, a polyethylene catheter (3 French size, 1.0 mm in diameter; Atom Co., Tokyo) was inserted into the right common carotid artery. Nine hundred microspheres (47.5±0.5 μm in diameter; NEN-005, purchased from New England Nuclear, Inc., Boston, MA, USA), suspended in 20% dextran solution, were injected into the right internal carotid artery through this cannula. After injection, the right common carotid artery was ligated and the wound was closed by sutures. Fifty-nine rats that underwent sham operation were injected with the same volume of vehicle without microspheres, and their right common carotid arteries were ligated. The non-operated (control) group was comprised of 19 non-operated rats.

Fifteen hours after the operation, the behavior of the operated rats was scored on the basis of paucity of movement, truncal curvature and force circling during locomotion, which are considered to be typical symptoms of stroke (12, 13). The score of each item was ranked from 3 to 0 (3, very severe; 2, severe; 1, moderate). The rats with >7 points were considered type A; 6–4, type B; and <4, type C. In the present study, we used only type A animals for the studies on metabolites of brain regions and cerebral infarct areas of the animal.

Determination of TTC-stained areas

In the first set of experiments, microsphere-injected, sham-operated and control rats were lightly anesthetized and decapitated at an appropriate experimental sequence. The brains were rapidly isolated and cooled on the ice. The brain was positioned on a brain holder and coronally sectioned 3, 5 and 7 mm from the frontal pole with razors. The sectioned brain tissue was incubated at 37℃ for 30 min with 2% 2,3,5-triphenyltetrazolium chloride (TTC) in physiological saline (14). The slices were then transiently immersed in a 10%-formalin solution, and they were photographed. The sum of TTC-stained, and TTC-unstained (including weakly stained) areas of three brain slices were estimated by a planimetric method.

Measurements of acetylcholine and choline

In the second set of experiments, the microsphere-injected, sham-operated and control rats were sacrificed at different time intervals after embolism with focal microwave irradiation to the head for 0.85 sec by a microwave applicator at 5.0 kW (model TMW-6402c, Muromachi Kikai Co., Tokyo). After decapitation, the head of the animal was immersed into liquid nitrogen and left for 10 sec. The cerebral hemispheres were isolated and separated on ice into three regions: cerebral cortex, striatum and hippocampus. After their wet weights were measured, each region was homogenized in 0.2 M $HClO_4$ and 0.01% of disodium ethylenediaminetetraacetate with a Polytron homogenizer (model PT-10, Kinematica, Lucerne, Switzerland) for 15 sec at the maximal speed. The extracting solution as described above contained 1 μM of ethylhomocholine as an internal standard for detection of choline and acetylcholine. After being left for 10 min at 0℃, the homogenate was centrifuged at 10,000×g for 15 min at 4℃. A part of the supernatant solution was taken and kept as a sample for determination of tissue amino acid content. Alternatively, the resultant supernatant solution was taken and neutralized with 2.5 M K_2CO_3. Then the resultant solution was centrifuged at 10,000×g for 15 min at 4℃. The supernatant solution was filtered through a membrane filter (0.45 μm), and the filtrate was sampled for determination of acetylcholine and choline contents.

Tissue acetylcholine and choline contents were determined by high-performance liquid chromatographic analysis. The extract was applied to a column for acetylcholine determination (Eicompak AC-gel + AC-enzymepak, Eicom, Kyoto) and eluted at 33℃ with a solution of 0.1 M Na_2HPO_4 containing 65 mg/l 1-decanesulfonic acid sodium salt, pH 8.5, at a flow rate of 1.0 ml/min (model EC-10, Eicom). The eluate was detected at 450 mV by an electrochemical detector (model ECD-100, Eicom).

Measurements of amino acid

The supernatant solution which had been kept as a sample for determination of amino acids was filtered through a membrane filter with a pore size of 0.45 μm (Advantec Toyo Inc., Tokyo) and then applied to an amino acid analyzer (model Hitachi-835, Hitachi, Tokyo). The amino acids were reacted with ninhidrin (835, Wako Pure Chemical Industries, Ltd., Osaka) and eluted by the solvent of the Ninhidrin reagent 835-set.

In the present study, the tissue metabolite contents are expressed as nmoles or μmoles/g frozen tissue. In a previous study (8), we examined the water contents of wet and frozen tissues of the cerebral cortex, striatum and hippocampus of microsphere-injected and sham-operated rats. Briefly, there were no significant differences in water content of the wet cortex, striatum and hippocampus of

the right and left hemispheres of the control and sham-operated rats. A slight increase in water content of the right hemisphere of the microsphere-injected rats was seen on the first and third days after the operation, but it was not statistically significant.

Statistics

The results are expressed as the mean±S.E.M. Statistical significance for the comparison of metabolic contents in the time course study was evaluated by analysis of variance, followed by Dunnett's *t*-test; and that for the comparison between two groups was performed by Student's *t*-test. The confidence value of more than 95% was considered to indicate a significant difference ($P<0.05$).

RESULTS

The ratios of the A-type, B-type and C-type animals produced by injection of microspheres were approximately 63%, 11% and 6%, respectively. Among the A-type animals, about 6% of the animals died within 3 days of the operation. The rest (20%) of the operated animals died by the 1st day after the operation. The results were similar to those of the previous study (8). The sham-operated rats showed no stroke-like symptoms and survived throughout the experimental period.

The time course of changes in the TTC-stained area is shown in Table 1. A significant decrease in TTC-stained areas of the right hemisphere was seen in the brain slices on the 3rd day after the embolism, and this decreased level was maintained until at least the 28th day. There were no changes in the TTC-stained areas of both hemispheres of sham-operated animals and the left hemisphere of microsphere-embolized animals throughout the experiment.

The time courses of changes in acetylcholine and choline contents of the cerebral cortex, striatum and

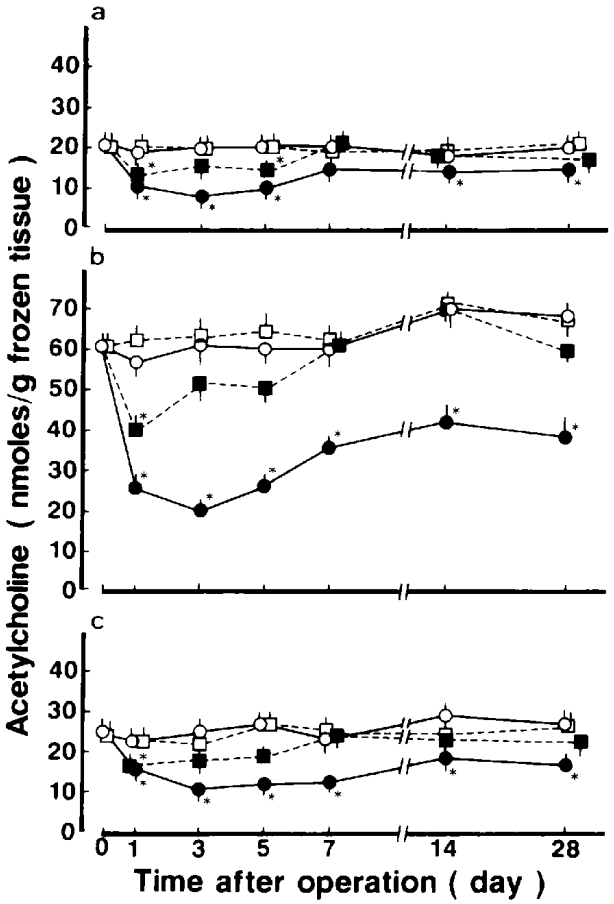

Fig. 1. The time course of changes in acetylcholine contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. (●) and (■) indicate metabolite contents of the above three brain regions of the right and left hemispheres in microsphere-injected rats, and (○) and (□) indicate those of sham-operated rats, respectively. Acetylcholine contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animal were 21.43±1.92, 61.48±3.22 and 25.00±0.98 nmoles/g frozen tissue (n=14), respectively. Each value represents the mean±S.E.M. of 8 (each day after the embolism) and 14 (control) experiments. *Significantly different ($P<0.05$) from the control value (0 day).

Table 1. Triphenyltetrazolium chloride-stained areas of brain slices from microsphere-embolized and sham-operated rats

Time after operation	Sham-operated animal		Microsphere-embolized animal	
	right	left	right	left
12 hr	222.1±1.9	225.9±1.8 (4)	196.0±15.0	233.3±4.5 (5)
1 day	213.6±1.9	212.8±2.3 (4)	215.6±22.8	213.4±4.5 (5)
3 day	219.6±1.9	219.3±2.4 (6)	46.4±12.6*	214.2±5.5 (5)
5 day	221.2±3.2	221.1±4.9 (4)	72.3±15.0*	221.9±4.8 (5)
7 day	221.9±2.7	221.4±2.8 (7)	58.5±13.3*	230.2±7.0 (5)
28 day	221.4±2.8	222.2±2.6 (6)	43.9±7.3*	218.8±5.9 (7)

Each values represents the mean±S.E.M. Numbers in parentheses indicate numbers of experiments. The pre-embolized (control) values of the brain slices of the right and left hemispheres are 212.7±1.8 and 212.2±1.7 mm^2 (n=5), respectively. *Significantly different from the corresponding value of sham-operated rats ($P<0.05$).

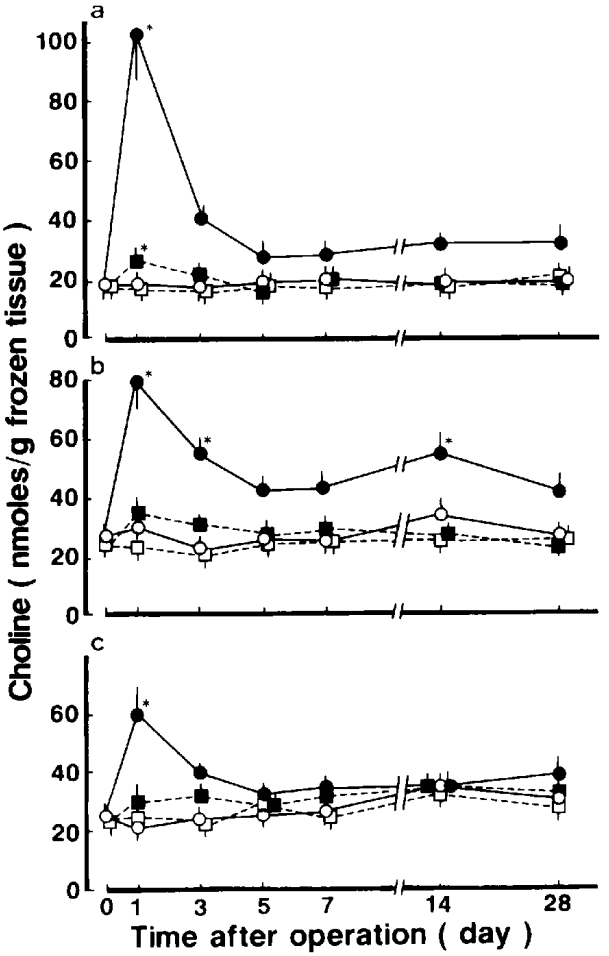

Fig. 2. The time course of changes in choline contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. Choline contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 18.61 ± 0.81, 27.09 ± 2.00 and 26.35 ± 1.58 nmoles/g frozen tissue ($n = 14$), respectively. Symbols and numbers of experiments are the same as those in Fig 1.

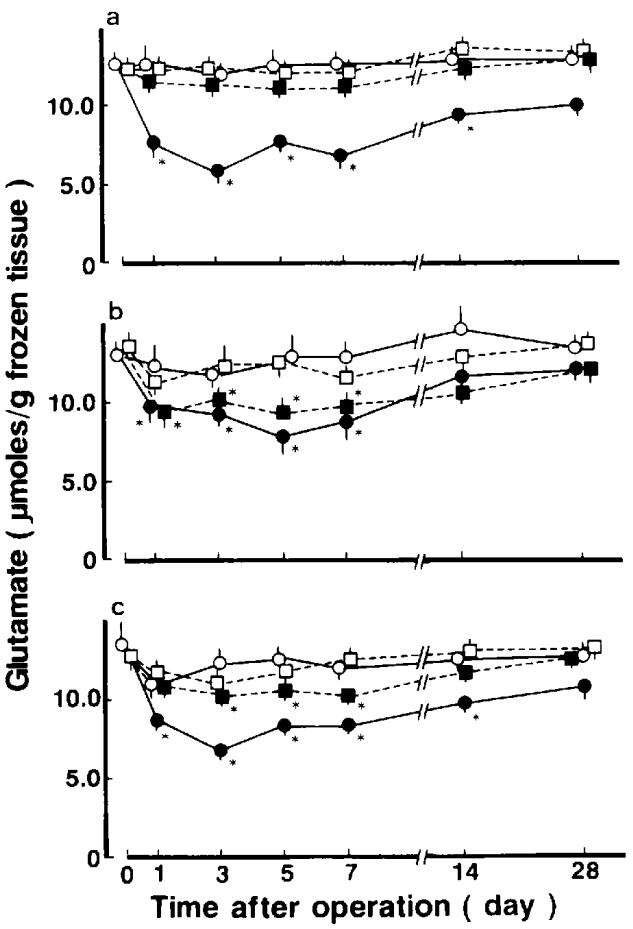

Fig. 3. The time course of changes in glutamate contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. Glutamate contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 12.46 ± 0.33, 13.08 ± 0.37 and 3.55 ± 1.32 μmoles/g frozen tissue ($n = 14$), respectively. Symbols and numbers of experiments are the same as those in Fig. 1.

hippocampus of both hemispheres with and without microsphere-embolism are shown in Figs. 1 and 2, respectively. In microsphere-injected rats, a marked reduction in acetylcholine content of these brain regions of the right hemisphere was seen on the first, 3rd and 5th days after the operation. After this, they tended to return slightly toward the initial values, but were still significantly lower than the control on the 14th and 28th days after the operation in all brain regions measured. The time course of changes in acetylcholine content of the cortex, striatum and hippocampus of the left hemisphere revealed a similar trend to that of the right hemisphere, but to a lesser degree. In sham-operated rats, tissue acetylcholine contents of both hemispheres were not altered at any period after microsphere-embolism.

In microsphere-injected rats, a marked increase in choline content of the three brain regions was seen one day after the operation. The cortical and hippocampal choline contents returned toward the initial levels by the 5th day, whereas the choline level of the striatum returned more slowly and remained high throughout the experimental period. In the left hemisphere, changes in choline content were marginal except for those of the cerebral cortex on the first day after the operation. In sham-operated rats, the choline content of both hemispheres was similar to the initial value throughout the experiment.

The time courses of changes in glutamate, GABA, aspartate, taurine and glycine contents of the cerebral cortex, striatum and hippocampus of both hemispheres with and without microsphere-embolism are shown in Figs. 3 to 7, respectively. In microsphere-injected rats, a sig-

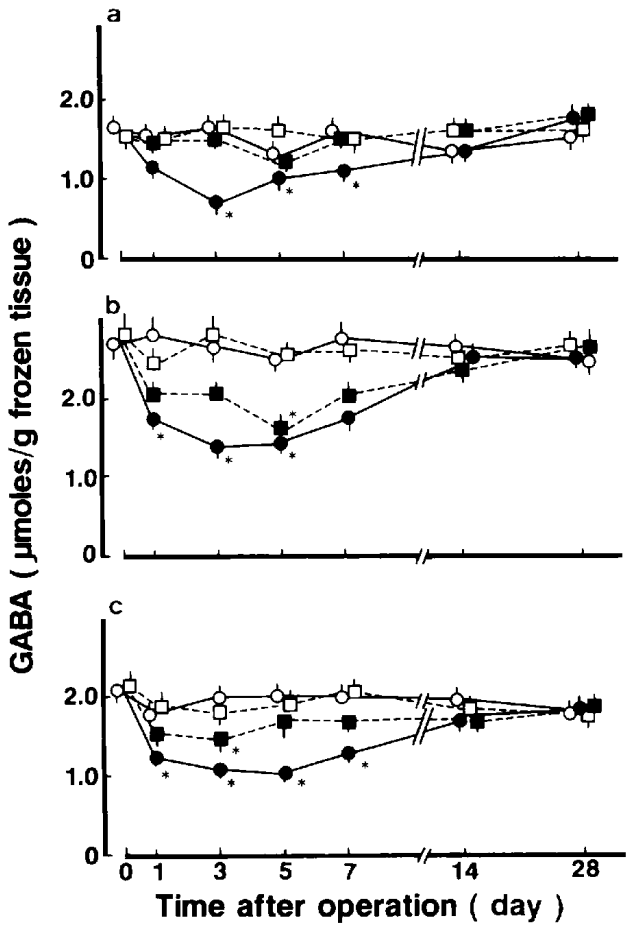

Fig. 4. The time course of changes in GABA contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. GABA contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 1.67±0.13, 2.68±0.19 and 2.09±0.13 μmoles/g frozen tissue (n=14), respectively. Symbols and numbers of experiments are the same as those in Fig. 1.

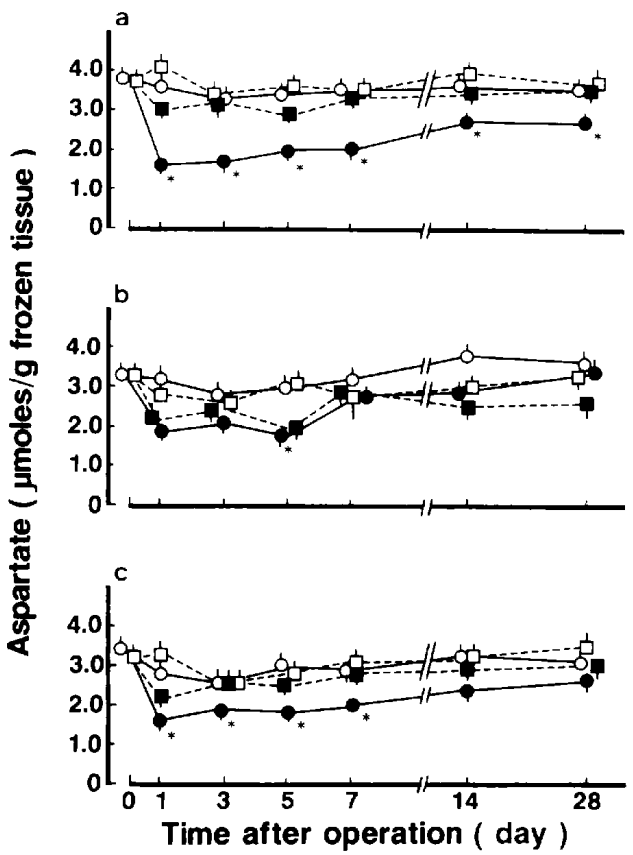

Fig. 5. The time course of changes in aspartate contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. Aspartate contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 3.78±0.13, 3.31±0.11 and 3.40±0.34 μmoles/g frozen tissue (n=14), respectively. Symbols and numbers of experiments are the same as those in Fig. 1.

nificant decrease in glutamate content of the three brain regions of the right hemisphere was seen throughout the experiment, except for in the striatum on the 14th and 28th days after the operation and both the cortex and hippocampus on the 28th day (Fig. 3). Glutamate content decreased most markedly on the 3rd day in the cortex and hippocampus and on the 5th day in the striatum. In the left hemisphere of microsphere-injected rats, the time course of changes in the cortical and hippocampal glutamate contents revealed a similar trend to that of the right hemisphere, but to a lesser degree. The extent of changes in the striatal glutamate content of the left hemisphere was similar, however, to that of the right hemisphere throughout the experiment. In sham-operated rats, the glutamate contents of the three brain regions of both hemispheres were similar to the control values throughout the experiment.

A marked decrease in GABA content of these three regions of the right hemisphere was seen in microsphere-injected rats from the first to 7th day after the operation, although statistically significant differences in the cortical GABA content were not detected on the first day and in the striatum on the 7th day after the operation (Fig. 4). Thereafter, GABA content returned almost completely to the initial level. In the left hemisphere of the microsphere-injected rats, both striatal and hippocampal GABA contents showed a trend to decrease throughout the experiment; significant decreases were detected on the 5th day in the striatum and on the 3rd day in the hippocampus. In sham-operated rats, the GABA content of the three regions of the right and left hemispheres was similar to the control throughout the experiment.

Both the cortical and hippocampal aspartate contents of the right hemisphere were markedly decreased through-

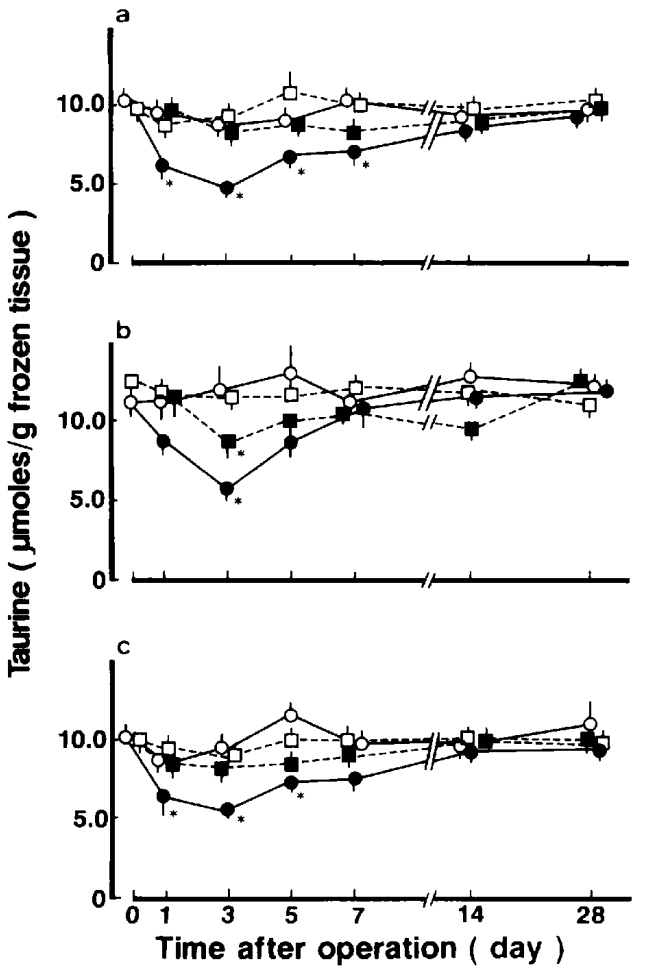

Fig. 6. The time course of changes in taurine contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. Taurine contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 10.17 ± 0.49, 11.32 ± 0.48 and 10.13 ± 0.77 μmoles/g frozen tissue ($n = 14$), respectively. Symbols and numbers of experiments are the same as those in Fig. 1.

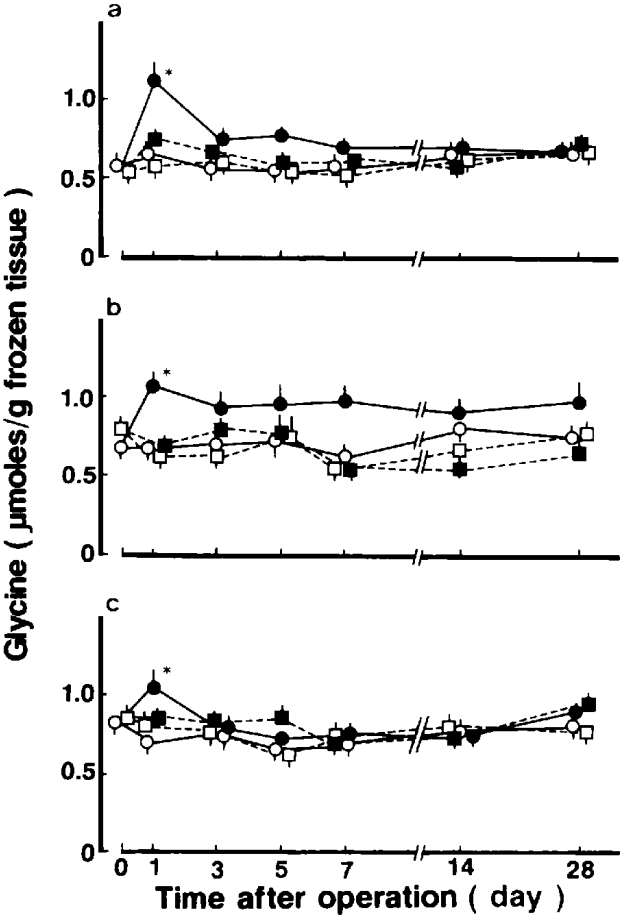

Fig. 7. The time course of changes in glycine contents of the cerebral cortex (a), striatum (b) and hippocampus (c) of the cerebral hemispheres after microsphere-induced cerebral embolism in rats. Glycine contents of the cerebral cortex, striatum and hippocampus of the right hemisphere of the control animals were 0.59 ± 0.03, 0.67 ± 0.05 and 0.82 ± 0.07 μmoles/g frozen tissue ($n = 14$), respectively. Symbols and numbers of experiments are the same as those in Fig. 1.

out the experiment and from the first to the 7th day after the operation, respectively (Fig. 5). The striatal aspartate levels of both hemispheres also tended to be decreased throughout the experiment, but a significant decrease was seen only in the right hemisphere 5 days after the operation. There were no significant decreases in aspartate content of the cortex, striatum and hippocampus of the left hemisphere throughout the experiment, although all these values were lower levels than the controls. In sham-operated rats, no significant changes in aspartate content were seen in either hemisphere or at any period of the experiment.

Taurine content was decreased after microsphere-embolism in all brain regions of the right hemisphere (Fig. 6). That is, the peak reduction in taurine content was seen 3 days after the operation. Significant decreases in taurine content were observed in the cortex of the right hemisphere from the first to 7th day after the operation and in the hippocampus from the first to 5th day, while the striatal taurine content was decreased significantly only 3 days after the operation. A significant decrease in the striatal taurine content of the left hemisphere was only seen 3 days after the operation. No appreciable changes in taurine content of these brain regions of both hemispheres of sham-operated rats were detected throughout the experiment.

The glycine content of these brain regions of the right hemisphere was increased on the first day after the operation (Fig. 7). Although the striatal glycine levels were slightly high thereafter, those of the cortex and hippocampus of the right hemisphere returned to the controls from

the 3rd to 28th day after the operation. The glycine levels of all brain regions of the left hemisphere were not significantly altered at any period of the experiment. Likewise, no change in glycine content was seen in any brain region of the right and left hemisphere of sham-operated rats.

The time courses of changes in serine and threonine contents of the cerebral cortex, striatum and hippocampus with and without microsphere-embolism were examined. Tissue contents of serine in the cerebral cortex, striatum and hippocampus of the right hemisphere of control animals were 1.30 ± 0.05, 1.41 ± 0.06 and 1.43 ± 0.16 μmoles/g frozen tissue; and those of threonine were 3.41 ± 0.39, 3.93 ± 0.39 and 3.60 ± 0.39 μmoles/g frozen tissue, respectively ($n = 14$). A temporary increase in serine and threonine contents of the three brain regions of the right hemisphere was seen on the first day after the operation. However, no significant alterations in the amino acids were seen throughout the experiment (details not shown). Likewise, there were no significant differences in serine and threonine contents of these brain regions of either hemisphere of the sham-operated rats throughout the experiment.

DISCUSSION

In the present experiment, we demonstrated a significant development of TTC-unstained areas in the microsphere-injected hemisphere, but not in the contralateral one, from the 3rd to 28 days after the embolism. The TTC-unstained area has been shown to be a convenient marker of the infarct area in the brain (14, 15). Thus, the results suggest that the microsphere-embolism employed in the present study induces long-term, possibly irreversible, cerebral ischemia. No further development of the TTC-unstained areas of the right hemisphere, at least, during one month was seen after the 3rd day, suggesting that microsphere-induced cerebral infarction is developed by this day to a greater degree.

In a previous study where the animals were subjected to the same experimental procedure as that in the present study, we observed a significant increase in tissue lactate and decreases in ATP and creatine phosphate contents of the cerebral cortex, striatum and hippocampus of rats during 28 days following the operation (8). Since an increase in tissue lactate and a decrease in tissue high-energy phosphates are typical metabolic indicators of tissue ischemia or oligemia (16–19), these results suggest that the brain regions examined in the present study are prone to ischemic insult for a period of at least 28 days after microsphere injection. In accord with this, we have observed a sustained decrease in the cerebral blood flow of these three brain regions, followed by cerebral infarction (20).

We used the microwave-irradiation and near-freezing method for determination of acetylcholine and choline contents in the brain regions. This method, characterized in a previous study (8), was found to provide acetylcholine and choline contents comparable to those reported in the literature (21, 22), suggesting that it is one of the methods relevant to the determination of labile brain metabolites.

We examined the time course of changes in acetylcholine and choline contents of the three regions of both cerebral hemispheres. As described earlier, these brain regions have been shown to be extremely sensitive to oligemia or ischemia (9–11). In the present study, we found a sustained decrease in acetylcholine content of these three brain regions throughout the whole experimental period monitored; in particular, the acetylcholine content of the striatum was profoundly decreased and was not fully restored even 28 days after the embolism. The results suggest that microsphere-embolism induces severe damage to acetylcholine synthesis and/or catabolism in the brain regions, particularly in the striatum. The vulnerability of acetylcholine content in the striatum to ischemia appears to be related to the high density of cholinergic terminals in this area, as evidenced by the presence of high concentrations of acetylcholine and high activities of choline acetyltransferase and cholinesterase (23). Several studies have demonstrated decreases in acetylcholine and increases in choline of brain regions following various ischemic insults induced by four vessel-ligation in rats (24), bilateral carotid artery ligation in Mongolian gerbils (25), and middle cerebral artery ligation in rats (26). Similarly, short periods of hypoxic insult induced by chemical hypoxia, anemic hypoxia, hypoglycemia and hypoxic hypoxia have also been shown to impair acetylcholine synthesis in vivo (27–29). Our results are consistent with these observations. In an experimental model, hypoxia-induced reduction of memory and judgment has been reported to be associated with a decrease in acetylcholine synthesis (30). These observations suggest an important role of acetylcholine content in brain function under normal and pathophysiological conditions. Thus, the observed marked decrease in acetylcholine contents of the three regions may also be indicative of severe damage to neuronal function after microsphere-embolism.

Acetylcholine is synthesized by choline and acetyl CoA, but de novo synthesis of choline is limited (31). It has been demonstrated, however, that there is no correlation between choline formation and the degree and distribution of cholinergic innervation (32, 33). It is well recognized that a rise in choline content upon ischemia is not attributable to the breakdown of acetylcholine, but rather results from hydrolysis of phospholipids (34). Choline content has been shown to increase after cerebral ische-

mia or hypoxia in vivo (26, 28) and in vitro (33, 34). The ischemia-induced increase in extracellular choline concentration is considered to be due to enhancement of its production from phospholipids and/or interference with its removal by the circulation (26). In accord with this, we found a marked increase in choline content of the cerebral cortex, striatum and hippocampus in rats following microsphere-embolism. The cortical choline content was most markedly increased one day after the embolism. This might be in agreement with the observations that the cerebral infarction, when roughly estimated by the TTC-staining method, is developed most markedly in the cortical region at an early stage of microsphere-embolism as compared to that in any other region (details not shown). The increase in choline content was sustained in the striatum much longer than in the other two regions. The decrease in acetylcholine of the striatum also lasted much longer than that of either the cerebral cortex or hippocampus. The results suggest that choline metabolism is not entirely related to the breakdown of acetylcholine in the embolism-induced ischemic area and that the striatum is most susceptible to the influence of microsphere-induced cerebral ischemia with regard to choline and acetylcholine content.

Amino acids are present in the brain in high concentrations and have been suggested to play a role as neurotransmitters or neuromodulators under normal as well as pathophysiological states. In the present study, we have observed long-term decreases in putative neurotransmitters, glutamate, aspartate, GABA after microsphere-embolism and relatively short-term changes in neuromodulators, taurine and glycine. The exact mechanism and significance of these changes in amino acid content can not be postulated from the results in the present study. There are conflicting results with respect to changes in cerebral amino acids following hypoxia and ischemia. For example, cerebral glutamate levels were found to be decreased in hypoxemia (35) and in acute hypoxia (36), but to be increased in ischemia (37, 38). Presumably, the difference is due to the different periods and degrees of ischemia or hypoxia induced. This also implies that we should be cautious about directly correlating changes in amino acid content during cerebral ischemia with the severity of pathophysiological alterations. There is increasing evidence that glutamate and aspartate, which are released in large quantities by cerebral ischemia, may exert neurotoxic effects (39–45). Furthermore, some investigators have postulated an involvement of excessive release of amino acids in delayed neuronal death in the ischemic brain (46, 47). The observed decreases in glutamate and aspartate in the present study probably can be attributed to sustained release of these amino acids following microsphere-induced cerebral ischemia.

Brain GABA content increases during a relatively short period of ischemia (38, 48) and hypoxia (30, 36, 49), and this is considered to be due to stimulation of glutamic acid decarboxylase and inhibition of GABA transaminase (38). In contrast, we have shown a marked decrease in cerebral GABA content in microsphere-embolized rats in the present study. The difference may be due to the severity and period of oxygen-deficiency induced.

Amino acid content of the brain regions following microsphere-embolism in the present study was altered more severely in the cerebral cortex than in the other two regions. Although the exact meaning of this phenomenon is not known at present, this appears to be related to the observations in a previous study (20) that the cortical blood flow was most markedly decreased after microsphere-embolism.

In summary, we demonstrated in the present study that microsphere-embolism in the brain, which mimics multifocal cerebral embolism, induces sustained damage to cerebral acetylcholine and neurotransmitter amino acid contents which may be indicative of severe impairment of neurotransmitter synthesis and/or catabolism in the brain. This model may be relevant to the examination of the efficacy of drugs which may improve the symptoms of, or limit the development of, cerebral ischemic diseases.

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