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Cerebral Embolization Impairs Memory Function and Reduces Cholinergic Marker Enzyme Activities in Various Brain Regions in Rats

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NARUMI, S., Y. KIYOTA AND A. NAGAOKA. *Cerebral embolization impairs memory function and reduces cholinergic marker enzyme activities in various brain regions in rats.* PHARMACOL BIOCHEM BEHAV **24**(6) 1729–1731, 1986.—Cerebral embolization was produced by injecting microspheres into the left internal carotid artery in rats. In these embolized rats, passive avoidance response was impaired at early stages after the embolization (3 days, 1 and 2 weeks), but 4 to 8 weeks later, recovery was achieved. A reduction in the activities of choline acetyltransferase (CAT) in the left side of the occipital cortex and hippocampus was observed in parallel with the impairment of learning behavior. These results suggest that impairments in the septo-hippocampal cholinergic pathways are likely to be closely related with the behavioral impairment in these rats.

Cerebral embolized rats Choline acetyltransferase Acetylcholinesterase Passive avoidance Hippocampus Cerebral cortex

ONE of the most serious clinical geriatric problems is the deterioration of memory and other mental processes. There are two known main types of dementia: dementia related with multiple-infarction and senile dementia of the Alzheimer type. Over 50% of dementia in Japan is the former. As cerebral embolized rats have memory impairment of passive or active avoidance response and water-filled multiple T-maze performance, they seem to be a useful model for studies on the multiple-infarction type of dementia [8]. In the present report, time-dependent changes in passive avoidance response and activities of cerebral cholinergic marker enzymes (choline acetyltransferase (CAT) and acetylcholinesterase (AChE)) were investigated 3 days and 1 to 8 weeks after cerebral embolization.

METHOD

Cerebral Embolization

Male Wistar rats (Japan Clea) weighing 250–300 g at the start of the experiment were used. The cerebral embolization was produced by injecting the microspheres (35±5 μm, 2,000 pieces) suspended in 20% dextran into the left internal carotid artery according to the method described by Kogure *et al.* [9] with a minor modification [7].

Passive Avoidance Task

Passive avoidance response was assayed by the procedure described in our previous paper [7]. Briefly, the acquisition trial in passive avoidance task was carried out 3 days, 1, 2, 4 and 8 weeks after the operation. The rats were individually placed in an illuminated compartment and allowed to enter a large dark compartment equipped with a grid floor. As soon as the rats entered the dark compartment, the door which separated the two compartments was closed and unescapable footshock (2.5 mA, 3 sec) was delivered through the grid floor. In the retention test performed 24 hr after the acquisition trial, the rats were again placed in the illuminated compartment, and the response latency to enter the dark compartment was measured. If the rat avoided entry longer than 300 sec, a ceiling score of 300 sec was assigned.

Enzyme Assay

Choline acetyltransferase (CAT, EC2.3.1.6) was assayed by the method of Fonnum [6]. Brain tissue was homogenized in 10 volumes of 0.32 M sucrose with a Polytron (setting 7 for 10 sec). The homogenates dissolved partially in 1% triton X-100 were used for enzyme preparation. The incubation mixture contained 50 mM sodium phosphate buffer (pH 7.4), 60 mM NaCl, 5 mM choline, 2 mM EDTA, 40 μM physostigmine and 50 μM 1-[^{14}C]-acetyl coenzyme A (0.02 μCi). After incubation at 37°C for 10 min, the reaction was stopped by adding 5 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.4) and 2 ml of acetonitrile containing sodium tetraphenylborate (5 mg/ml). The reaction mixture was trans-

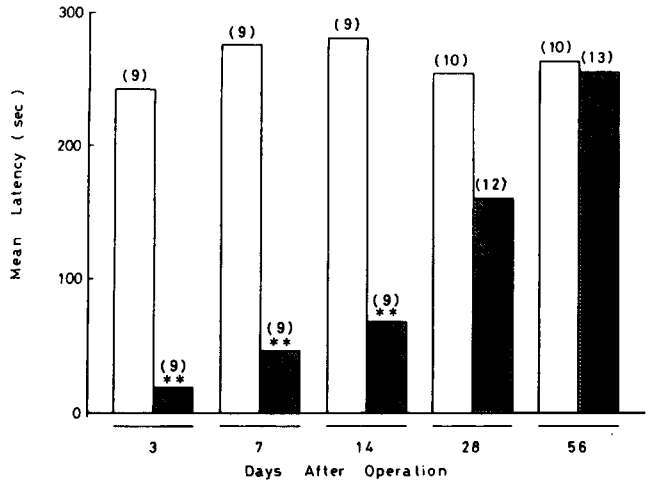

FIG. 1. Passive avoidance response at various times after cerebral embolization in rats. The embolization was produced by injection of 2,000 microspheres into the left internal carotid artery and the acquisition trial was carried out 3 days, 1, 2, 4 and 8 weeks after the operation. Different groups of rats were used in each retention test. The retention test was performed 24 hr after the acquisition trial. Number of rats is shown in parentheses. $^{**}p<0.01$ vs. sham operated group (U-test). Open column: Sham operated group, shaded column: Cerebral embolized group.

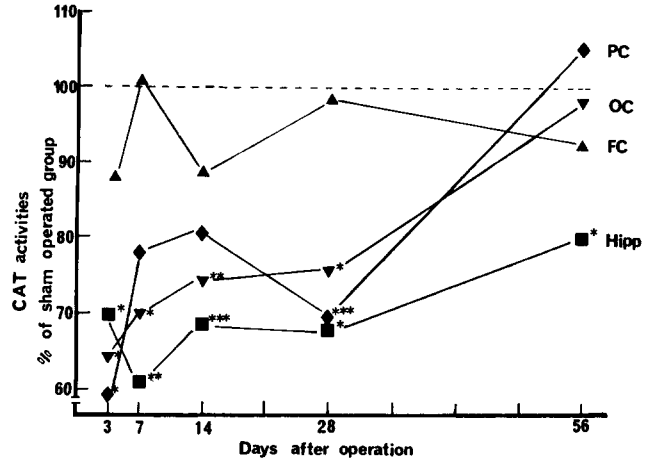

FIG. 2. Choline acetyltransferase (CAT) activities in various brain regions obtained at various times after cerebral embolization in rats. The embolization was produced by injection of 2,000 microspheres into the left internal carotid artery. All brain regions were obtained from the left hemisphere. The activities in the cerebral cortex and hippocampus of sham operated group was 250–400 and 300–450 pmol/mg protein/min, respectively. The activities in the right hemispheres in the embolized and sham operated rats were almost the same. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. respective sham operated group (t-test). N=6. ◆: parietal cortex (PC). ▼: occipital cortex (OC), ▲: frontal cortex (FC), ■: hippocampus (Hipp).

ferred to a plastic vial, into which toluene scintillation mixture was added. After the mixture was shaken, radioactivity was counted in portions of the organic phase using an Aloka liquid scintillation spectrometer. Acetylcholinesterase (AChE, EC 3.1.1.16) was assayed by the method of Reed *et al.* [11]. ATPase activity (ATP phosphohydrolase, EC 3.6.1.3.) was assayed in a medium containing 5 mM disodium ATP, 5 mM $MgCl_2$ and 100 mM Tris-acetate buffer (pH 7.5). The mixture, in a volume of 1 ml, was incubated at 37°C for 20 min and the enzymatic reaction was terminated by adding 0.5 ml of an ice-cold 5% trichloroacetic acid solution. The inorganic phosphate liberated during the incubation was estimated by the method of Takahashi [13]. The following drugs and analytical grade of reagents were used: 1-[^{14}C]-acetyl coenzyme A, 1-[^{14}C]-acetylcholine, carbon microsphere (New England Nuclear), physostigmine salicylate (Merck), ACS® II (Amersham), adenosine-5′-triphosphate disodium (ATP, Boehringer Mannheim), sodium tetraphenylborate, ethylenediamine tetraacetic acid (EDTA, Dojin Yakukagaku), dextran, choline chloride, acetylcholine chloride (Sigma).

Statistical comparison between different treatments was made using Student's t-test and Mann-Whitney U-test (two-tailed).

RESULTS

The results of the single trial passive avoidance response examined in the embolized and sham operated rats (control group) are shown in Fig. 1. The avoidance response in the embolized rats was impaired at the early stages after the embolization (3 days, 1 and 2 weeks), but this impairment was overcome 4 to 8 weeks later. The control rats showed a good response during the experimental period.

The activities of CAT in the left side of the occipital cortex and hippocampus, ipsilateral to the microspheres injected side, were significantly decreased to 60–70% of the control group from 3 days to 4 weeks after the cerebral embolization. The decrease in CAT activity in the cerebral cortex was recovered 8 weeks after the operation, but the reduction in the hippocampus remained at the levels of 80% of the control (Fig. 2). The activity in the parietal cortex was decreased only at 3 days and 4 weeks, and that in the frontal cortex was not altered by the embolization.

The activity of AChE changed in a manner seen in the CAT activity, but the recovery of AChE was slightly more rapid than that of CAT (data not shown). The time-dependent changes in the cholinergic marker enzymes paralleled those in the passive avoidance responses.

The activity of Mg^{2+}-dependent ATPase (Mg^{2+}-ATPase) in the hippocampus did not differ between the control and cerebral embolized rats at each test point (data not shown), thereby suggesting specific changes in cholinergic marker enzymes.

DISCUSSION

Major cholinergic input to the cerebral cortex and hippocampus originates in the basal forebrain (the nucleus basalis of Meynert) and in the medial septum in primates, respectively [4]. In cerebral embolized rats, the latter cholinergic pathway seemed to be impaired, because the activities of CAT and AChE were reduced mostly in the occipital cortex and hippocampus. From the kinetic analysis using a double-reciprocal plot, the reduction of CAT activity in the hippocampus was due to a reduction in the enzyme levels (Vmax), but not the affinity (Km).

Pharmacological and neurochemical studies suggest that the brain cholinergic system plays a major role in memory function in both humans and animals [1, 3, 5, 10, 12]. A marked decrease in CAT activity is observed in a widespread cortical area or the hippocampus in patients afflicted with Alzheimer's type of dementia [1, 2, 10]. In the present study,

it was demonstrated that CAT activity in the cortex, especially in the occipital cortex and hippocampus was markedly decreased in the embolized rats. Thus, in the vascular type of dementia, the brain cholinergic hypoactivity may be responsible, at least in part, for the disturbed memory function.

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