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Molecular Forms of Hippocampal Acetylcholinesterase and Their Changes Following Septal Lesions in the Rat

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Summary

Changes of acetylcholinesterase activity and its molecular forms, extracted by Triton X-100 and separated by polyacrylamide gel electrophoresis, were studied in the rat hippocampus following septal lesions. Detection of acetylcholinesterase was made densitometrically. While the total activity of acetylcholinesterase was decreased, its molecular forms exhibited a different pattern of changes: the heavy forms were decreased, while the light ones were increased. The results support the view that different acetylcholinesterase molecular forms serve different regulatory mechanisms.

Key words

Acetylcholinesterase – Hippocampus – Molecular forms – Septal lesions – Rat

Introduction

The demonstration of enzyme changes in some parts of the brain following lesions of defined brain structures represents a worthwhile approach to the study of different projections between various brain regions. Using this methodology, cholinergic projections were studied by determination of cholinergic markers such as acetylcholinesterase (AChE, EC 3.1.1.7) and choline acetyltransferase (CAT, EC 2.3.1.6) (Paxinos and Butcher 1985). Although CAT is considered to be a better marker of cholinergic innervation, AChE activity has frequently been used to demonstrate cholinergic pathways in the brain of different species (Bajgar *et al.* 1977, Herink *et al.* 1975, Paxinos and Butcher 1985). Moreover, the relationship between hippocampal AChE activity and the cholinergic septohippocampal connections was demonstrated by histochemical detection of the loss of AChE activity (Shute and Lewis 1961, 1965); the distribution of CAT immunoreactive fibres and terminals were found to correspond to the distribution of histochemical AChE activity (Zimmer *et al.* 1985, 1986).

It appears from our previous results concerning AChE determinations in different parts of the brain following septal lesions (Bajgar *et al.* 1977, Herink *et al.*

1975) that the hippocampus is connected with both the dorsal and medial septal nuclei.

However, these studies mostly described total AChE activity despite the existence of different molecular forms of AChE in the rat brain (Bajgar 1979, Lenz and Maxwell 1981, Skau 1986, Rakonczay 1988). Oderfeld-Nowak and Skangiel-Kramska (1976) showed alterations of the isoenzymic pattern in the hippocampus following septal lesions of the rat medial septum. The changes were observed in the membrane-bound but not in the soluble fraction of the enzyme. In addition to the two slow-migrating electrophoretic bands seen in the membrane-bound AChE fraction of the normal hippocampus, a fast-migrating enzyme band became visible after denervation.

The aim of the present report was to study the dynamics of changes in AChE activity and its molecular forms in the hippocampus produced by lesions of the medial septum.

Material and Methods

Male albino rats (VELAZ Praha) weighing 170–200 g were used in our experiments. The animals were randomly divided into groups, each comprising 6 animals. The control group was given only thiopental anaesthesia (50 mg/kg, intraperitoneally) without any surgical procedure. In the animals of the sham-operated group, an electrode was placed under thiopental anaesthesia above the septal area for 30 s without passing any current. In the experimental animals, small electrolytic lesions were produced in the medial septal area under the same anaesthesia using a stereotactic instrument. Lesions were made by passing direct current (0.2 mA) for 30 s through stainless steel electrodes insulated except for the tips. The location of the electrodes (coordinates: AP 0.75 mm anterior to the bregma, L 0 mm, V 5.5 mm) was controlled by histological verification (Herink *et al.* 1975).

The rats were killed by exsanguination from the carotid artery and the brains were removed and frozen. AChE activity and its molecular forms were determined in the hippocampus. The sample for AChE activity determination was prepared as follows: the dissected hippocampus was homogenized (1 : 10) with 0.5 % Triton X-100 (Koch Light Lab., England) and centrifuged for 60 min at 105 000 x g and 4 °C (MSE, 50 T.C., England). The obtained homogenates and supernatants were used for total AChE activity determination. AChE activity was determined according to Ellman *et al.* (1961) and it was expressed as μmol of substrate (acetylthiocholine) hydrolyzed per min per g wet tissue weight (ml of the sample) or as percentage of the controls. Electrophoresis of the supernatants (40 μl) was carried out in 7.5 % polyacrylamide gel for 60 min at 240 V and 40 mA. AChE activity in the gel was detected histochemically using acetylthiocholine iodide (Lachema Brno, Czechoslovakia) as substrate. The method is based on the interaction of hydrolytic product thiocholine with copper and copper thiocholine is converted to copper ferrocyanide (Hatchett's brown). Following detection, the gels were scanned on a Vitatron densitometer (Sci. Instr., Eafde, Netherlands) and the activity was expressed in integrational units corresponding to the area under the densitometric curve or as percentage of the controls (Bajgar 1979).

The results were statistically evaluated by Bartlett's test (homogeneity of the data) and differences between groups were determined by analysis of variance using a Hewlett-Packard 9830 A programmed calculator.

Results

Normal AChE activity in homogenates of the hippocampus in the control group was 6.1 ± 0.5 μmol of substrate hydrolyzed/min/g tissue wet weight. The activity in the samples for electrophoretic separation was 81.3 ± 10.5 % of activity of the whole homogenate. Electrophoretic separation showed that AChE activity can

be separated into four bands designated by arabic numerals *1* – *4*. Relative activities of these forms were determined in the control group as follows: *form 1* (highest electrophoretic mobility) 7.8±4.1 %, *form 2* 12.2±7.2 %, *form 3* 34.9±12.3 % and *form 4* (lowest electrophoretic mobility) 45.1±10.2 %, respectively.

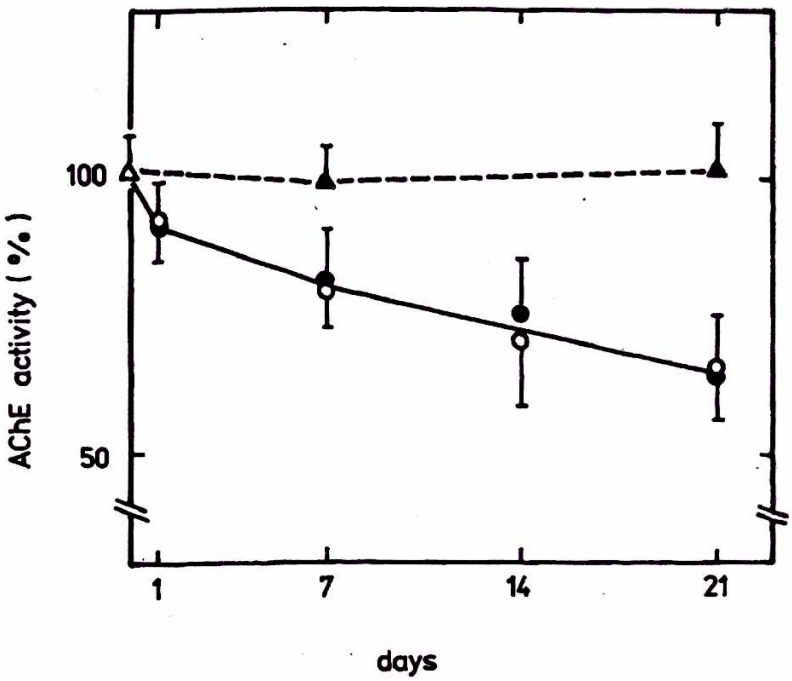

Fig. 1
The changes of AChE activity in the hippocampus following septal lesions in rats. Open triangles – control group (without surgery); closed triangles – sham-operated group; open circles – group with septal lesion (results from direct determination of AChE activity): closed circles – group with septal lesion (values calculated from determinations of AChE molecular forms). Each point represents the mean of 6 measurements and bars are 95 % confidence limits. Control AChE activity (100 %): 4.69 ± 0.64 μmol/min/ml.

Following septal lesions AChE activity in sham-operated animals remained unchanged 1, 7 and 21 days after the operation. However, a decrease of AChE activity in the hippocampus was observed at different time intervals after the lesions. These changes were significant ($p<0.05$, F test) (Fig. 1). AChE molecular forms were changed in two opposite ways: forms with lower electrophoretic mobility (designated *4* and *3*) were decreased and forms with higher electrophoretic mobility (designated *1* and *2*) were increased (Fig. 2) The decrease of activity of the various AChE forms was most marked for the form with the lowest electrophoretic mobility; the increase was highest for the form with highest electrophoretic mobility. About 20 % of total activity (i.e. *forms 1, 2*) was increased and the remaining forms (i.e. *forms 3, 4*) was decreased. Experimentally determined total AChE activity (representing the activities of all molecular forms) was decreased. Thus, the calculated sum of activities of the forms corresponds to the experimentally obtained results on total AChE activity (Fig. 1).

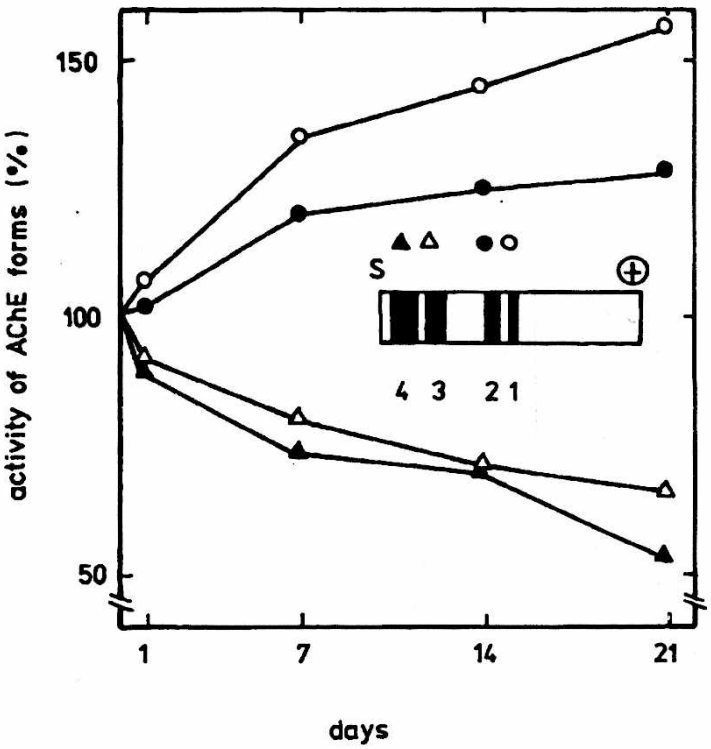

Fig. 2
Schematic representation of AChE molecular forms in the hippocampus and their changes following septal lesions. Each point represents the mean value of 6 measurements. Standard errors varied from 12 to 18 %. Distribution of activities of AChE forms (expressed in integration units representing the area under the densitometric curve) in experimental groups 1 and 21 days after the operation, respectively, are following: form 1 (75 ± 9.2; 110 ± 19.8), form 2 (132 ± 16.5; 168 ± 25.2), form 3 (318 ± 38.4; 235 ± 30.5), form 4 (400 ± 52.5; 240 ± 28.9).

Discussion

The determination of total AChE activity following lesions of various regions of the brain is one of the usual methodical approaches for demonstration of cholinergic projections in the brain. It has not been excluded in studies demonstrating diminished AChE activity in different parts of the brain induced by septal lesions that the changes of AChE molecular forms might be different as compared with total AChE activity. The multiplicity of AChE molecular forms has been shown by a variety of different methods (Siek *et al.* 1990, Rakonczay 1988, Skau 1986, Bajgar 1979). There are two main methods of separation – electrophoresis based on molecular weight and electric charge of the AChE forms, and density gradient centrifugation based on molecular weight only. The comparison of both methods showed that the heavy electrophoretic form in the human brain corresponds to the G-4 AChE form (Siek *et al.* 1990). The G-4 form was found to be preferentially localized presynaptically in the membrane, but not in the cell body (Siek *et al.* 1990).

The heavy form was decreased in our experiments. This observation suggests that this form, representing the membrane pool of AChE, is degraded following

interruption of cholinergic fibres. The fast-migrating fraction represents the light molecular form of AChE (Oderfeld-Nowak and Skangiel-Kramska 1976) and probably corresponds to the newly synthesized AChE. Inhibition studies with soman (Lenz and Maxwell 1981) and DFP (Michalek *et al.* 1981) also showed that low molecular weight forms of AChE are the first steps in the biosynthesis of the enzyme molecule. Following septal lesions, degeneration of cholinergic terminals in the hippocampus was accompanied by a decrease of the heavy form. This heavy form of AChE was the most sensitive to the administration of irreversible cholinesterase inhibitors (Meneguz *et al.* 1989, Lenz and Maxwel 1981). In connection with other observations on the physiological role of AChE forms (Bajgar 1979, Rakonczay 1988), our results support the view that different AChE molecular forms may play a different physiological role and that the heavy form might be of importance for cholinergic transmission.

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