QUANTITATIVE HISTOCHEMICAL DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY IN LAYERS OF THE RAT HIPPOCAMPUS

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KEY WORDS: hippocampus; quantitative histochemistry; maps of distribution of acetylcholinesterase activity; seasonal differences.

A positive acetylcholinesterase (AChE) reaction in the hippocampus has been shown to be produced by nerve cells and their processes which form connection with cholinergic septo-fimbrial afferents [3, 5, 8, 13, 16]. Until now the histochemical AChE reaction in the rat, rabbit, guinea pig, and human hippocampus has been assessed mainly visually [4, 8, 9, 12, 14]. However, the end products of histochemical reactions can be measured densitometrically [1] and microchemically [15]. Quantitative estimation of AChE activity has so far been carried out only in layers of area CA1 of the rabbit and rat hippocampus [2, 11, 17].

The object of this investigation was to test a simple and readily available visual ranking method [6, 7] for comparing maps of the laminar distribution of AChE activity in the hippocampus suitable for use in objective comparisons. The following aims were pursued: 1) to draw a map of the distribution of AChE activity in all layers of areas CA1, CA2, and CA3 of the rat hippocampus and to compare it with maps previously published; 2) to compare the laminar distribution of AChE activity in area CA2 of the rat hippocampus in different seasons of the year.

EXPERIMENTAL METHOD

Eight intact noninbred male albino rats weighing 160-190 g were used. Frontal brain sections 10 μ thick were cut on a freezing microtome and stained by the Karnovsky–Roots method for AChE [10]. Diisopropyl fluorophosphate (DFP) in a concentration of 10^{-6} M was used as inhibitor. Some of the animals were decapitated in the spring (three rats), some in the fall (three rats), and some in the summer (two rats). The intensity of the AChE reactions was determined in areas CA1, CA2, and CA3 of the right dorsal hippocampus in the following layers: Str. alveus et oriens (in the outer and subpyramidal zones, and in CA1 in the inner zone also), Str. pyramidale, Str. lucidum (in CA2 and CA3), Str. radiatum (in the infra-pyramidal and inner zones), Str. lacunosum, and Str. moleculare. AChE activity was estimated as follows. A grid with 10 squares (probes) was introduced into the ocular of the microscope. The probes were equal in size and small enough for the area of the field of vision covered by the probe to be uniform in optical density (magnification 20 × 7). The investigator picked out five gradations of optical density from minimal to maximal observed in the given preparation in the course of preliminary training, and numbered them from 0 to 4 (visual ranking method [6, 7]). The ranks of the densities in 10 probes in a random field in the given layer were added together and formed the first term of a cumulant. The sum of the ranks of three fields gave the third step of the cumulant, and so on, as far as the 10th step. Cumulants of this sort were drawn up for each layer of each of the three areas of the hippocampus. Maps of the distribution of AChE activity were drawn by summation of the steps of these cumulants (from the first to the 10th) for the five rats decapitated in the summer

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TABLE 1. Maps of Laminar Distribution of AChE Activity (in %) in Areas of Rat Hippocampus during Summer and Fall (A) and in Area CA1 (B) of the Rat (I and II) and Rabbit (III) Hippocampus

Layer of hippocampus	A			B		
	CA1	CA2	CA3	I	II	III
Str. alveus	19	21	17	28	20	39
Str. oriens:						
outer zone	58	56	41	86	70	75
middle zone	35			52		59
subpyramidal zone	68	100	66	100	100	100
Str. pyramidale	39	64	57	57	134	53
Str. lucidum		25	26			
Str. radiatum:						
infrapyramidal zone	49	49	47	72	92	88
inner zone	13	19	17	19	37	52
Str. lacunosum	46	54	54	68	54	103
Str. moleculare	19	38	35	28	42	96

Legend. AChE activity in subpyramidal zone of area CA2 (in part A) and area CA1 (in part B) taken as 100.

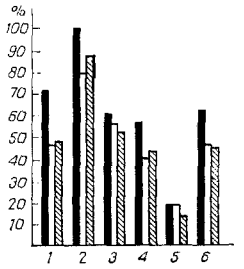

Fig. 1. Distribution of intensity of AChE reaction in layers of area CA2 of rat hippocampus in spring (black columns), summer (unshaded columns), and fall (obliquely shaded columns). Abscissa, layers: Str. oriens (1 — outer, 2 — subpyramidal zones), Str. pyramidale (3), Str. radiatum (4 — infrapyramidal and 5 — inner zones), and Str. lacunosum (6); ordinate, intensity of AChE reaction (in %). Intensity in subpyramidal zone of Str. oriens in spring rats taken as 100.

and fall. The values of the cumulants at all steps for the subpyramidal part of Str. oriens of area CA2 were taken as 100. All other cumulants were expressed as percentages of this value. The map was drawn on the basis of ratios of the 10 steps of the cumulants thus obtained, but ratios of a few earlier steps also were calculated in order to ensure stability of the ratios thus calculated. To assess seasonal differences in the intensity of the AChE reaction between homonymous layers, cumulants of these layers were compared for area CA2 of the hippocampus of rats killed in the spring, summer, and fall. Seasonal maps of distribution of AChE activity were drawn as described above. The value of the cumulant for the 10th step of the subpyramidal zone of Str. oriens of the spring map was taken as 100 and

all other cumulants for the 10 steps of the three maps were expressed as percentages of it. The three maps were compared on the basis of these percentages.

EXPERIMENTAL RESULTS

Parameters of the maps of laminar distribution of AChE activity in areas CA1, CA2, and CA3 of the rat hippocampus in the summer and fall are given in Table 1 (part A); part B gives parameters obtained for area CA1 of the rat hippocampus by visual ranking (I, our own data) and microchemical (II, [17]) methods, and also obtained in rabbits by a microdensitometric method (III, [2]).

All values in part A were taken for the 10th step of the cumulant. The stability of the values used is illustrated by the following example: The ratios between the 1st, 2nd, ..., and 10th steps of the cumulants for the outer and subpyramidal zones of Str. oriens for area CA2 are given by the following series of numbers: 52, 52, 54, 55, 55, 55, 56, 56, 56, and 56%. It will be clear from these figures that after small fluctuations in the early steps the ratios between the two cumulants stabilized after the 7th step at a steady level (56%). Since the cumulants became increasingly representative and stable with each consecutive step, the probability that this ratio could change in subsequent steps is extremely small.

Quantitative estimates of intensity of the AChE reaction (in conventional units) in layers of area CA1 of the rat hippocampus obtained by microchemical analysis [17] and in the rabbit hippocampus calculated by a microdensitometric method [2] are given in the literature. Our own results for area CA1 (visual ranking method) are compared in part B of Table 1 with the results of these investigations. This comparison shows that the relative percentages of AChE activity in the layers of area CA1 of the rat hippocampus obtained by the visual ranking method and by microchemical analysis are sufficiently close. However, the values for Str. pyramidale in the second case are very much on the high side. The reason is that neighboring infra- and subpyramidal zones were inevitably partly included in the specimen. Comparison of our own results with the corresponding densitometric data obtained in rabbits [2] shows good agreement between the ratios for AChE activity from Str. alveus to the infrapyramidal zone of Str. radiatum, but in the deep layers AChE activity in the rabbit is much higher than in the rat, and there is likewise not the same contrast between the activity of the different layers reflected in the visual investigations [4, 15]. The results obtained by the different methods of quantitative histochemical analysis thus agree satisfactorily.

The intensity of the AChE reaction in layers of area CA2 of the rat hippocampus in the summer and fall is shown in Fig. 1. Ratios of intensity between the different layers were practically identical in all seasons. The intensity of the AChE reaction was highest in spring, and rather lower and equal in the summer and fall. Only in Str. pyramidale and the inner zone of Str. radiatum did AChE activity remain unchanged season by season.

The suggested method of quantitative estimation of intensity of the AChE reaction in histochemical preparations thus gives reproducible results. A map of laminar distribution of AChE activity in areas CA1, CA2, and CA3 of the rat hippocampus in the summer and fall was drawn on a quantitative basis and it showed that the intensity of the AChE reaction in area CA2 of the rat hippocampus reaches a maximum in the spring, and is somewhat lower in the summer and fall, and the ratios between the different layers remain constant in all seasons.

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MORPHOLOGICAL DIFFERENCES BETWEEN ERYTHROCYTES OF ARTERIAL AND VENOUS RAT BLOOD REVEALED BY SCANNING ELECTRON MICROSCOPY

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Because the blood shares a common embryogenetic origin with a wide range of tissues derived from the mesenchyme, by using erythrocytes to model various pathological states and processes at the membrane level it is possible to elucidate general principles characteristic of the cell membranes of different organs and tissues.

The most adequate method of studying the shape and surface of erythrocytes at the present time is scanning electron microscopy. Data on normal rat erythrocytes obtained by methods of optical microscopy are inadequate [7] and at times contradictory [8]. Meanwhile morphological characteristics of erythrocytes obtained from arterial and venous blood are not to be found in the literature.

This paper gives a quantitative description of forms of erythrocytes obtained from arterial and venous rat blood studied by scanning electron microscopy.

EXPERIMENTAL METHOD

Fifteen noninbred male rats weighing 200-300 g were used. Under pentobarbital anesthesia blood was taken into a syringe with 2% glutaraldehyde from the bifurcation of the abdominal aorta and from the portal vein. No anticoagulants were used.

The blood was prefixed in 2% glutaraldehyde for 1 h, then centrifuged (1000 rpm) for 5 min, after which the residue was washed twice in 0.1 M phosphate buffer, pH 7.4. The cells were fixed in 1% OsO_4 for 1 h. They were then washed once and dehydrated in acetone of increasing concentration from 40 to 90%, and 3 times in 100% acetone, for 15 min in each case. One drop of fixed and dehydrated erythrocytes was applied to a support previously treated with 0.1 N HCl, acetone, and ether. The preparations were dried in air at room temperature (18-20°C) and sprayed with gold in a vacuum spray. The preparations thus obtained were studied in a Hitachi S-500 (Japan) scanning electron microscope. To obtain quantitative characteristics of the two forms of erythrocytes 400 cells (200 from the artery, 200 from the vein) from each animal were counted and the results subjected to statistical analysis.

EXPERIMENTAL RESULTS

Pictures of erythrocytes from the arterial and venous blood of normal animals obtained in this way gave a sufficiently complete idea of the shapes of the erythrocytes and details of their surface, so that the following classification could be suggested.

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