A Comparative Study of Cholinergic Systems of the Neocortex and Hippocampus in Rats with Low and High Resistance to Hypoxia

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Synaptic structures in the neocortex and hippocampus of the intact brain were compared between rats with low and high resistance to hypobaric hypoxia. Activities of choline acetyltransferase, acetylcholinesterase, Na,K-ATPase, and the portion of protein in the light and heavy synaptosome fractions and subfractions were measured. A discrepancy in cholinergic metabolism molecular mechanisms between high and low resistance animals have been found in the heavy somatosoma fraction from the neocortex. Activities of choline acetyltransferase, acetylcholinesterase, and Na,K-ATPase in the synaptolemmal subfraction of low resistant rats were much lower than in high resistant rats. This implies a less effective synaptic transmission in proper cholinergic neurons in the low resistance animals and, therefore, specifically changed neuron functioning in the circulation control. No differences in the cholinergic components of either neocortical light synaptosome fraction or hippocampal light and heavy synaptosome fractions were found between low and high resistance rats.

Key Words: *resistance to hypoxia; neocortex; hippocampus; synapses; synaptosomes; synaptic membranes; synaptoplasm; choline acetyltransferase; acetylcholinesterase; Na,K-ATPase*

In clinical literature, severe postischemic memory and cognitive disordes associated with damage of certain groups of neurons in the hippocampus and neocortex have been described [13]. Recently, some indications appeared that the cholinergic mechanisms play a leading role in such pathological processes. For instance, a postischemic inhibition of cholinergic transmission preceding the death of pyramidal neurons have been demonstrated in the gerbil hippocampus [16]. However, little is known about the genesis of cholinergic structures sensitive to ischemia, as well as about the mechanisms responsible for implicating these structures in hypoxia pathogenesis and their role in individual brain sensitivity to ischemia.

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In the present work, we studied acetylcholine metabolism in nerve terminals in neocortical and hippocampal synaptosomes in intact rats with different resistance to hypoxia. Cholinergic synaptosomes from the light (C) fraction belong to neurons of the forebrain large cellular nucleus basalis, whereas those from the heavy fraction (D) belong to neurons of the neocortex [3]. So, the cholinergic nerve terminals from fraction C and D should be investigated separately. The state of cholinergic metabolism was determined on the basis of activities of enzymes of acetylcholine synthesis and catabolism: choline acetyltransferase (ChAT) and acetylcholinesterase (AchE). Several molecular forms of these enzymes are known. Dividing of synaptosomal fractions into subfractions allows one to separately investigate the membrane- and cytosol-associated forms of the enzymes (m-ChAT, m- AchE, c-ChAT, c-

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AchE, respectively), which are functionally different [14,15].

MATERIALS AND METHODS

Experiments were performed on random-bred albino rats weighing 250-350 g. The rats were divided into low-resistant (LR) and high-resistant (HR) to hypoxia according to thei resistance to hypoxia test (lifting to a height of 11,000 m at a rate of 180 m/sec) [5]. Such a division is based on the indications that functional and metabolic characteristics of these neurons in LR and HR rats differ considerably [9]. Three and five weeks after the test, the animals were decapitated. Fractions of C and D synaptosomes were separated from the neocortex and hippocampus on sucrose gradient. Then subfractions of synaptic membranes were separated at the boundary between 0.6-1.0 M sucrose and the entire synaptoplasm [1]. The fractions were kept at -26°C. We used autoradiography to determine ChAT and AchE activities in C and D fractions [8,10], substrate-regenerating method for Na,K-ATPase activity [12], and Lowry method for protein measurements. Since Na,K-ATPase is a plasma membrane protein, it was absent from the synaptoplasmic fraction. We determined ChAT and AchE activities both in the synaptolemmic (m-ChAT, m- AchE enzyme forms) and synaptoplasmic fraction (c-ChAT, c-AchE forms). The data were measured in units of enzyme activity (protein portion) per gram tissue and statistically analyzed.

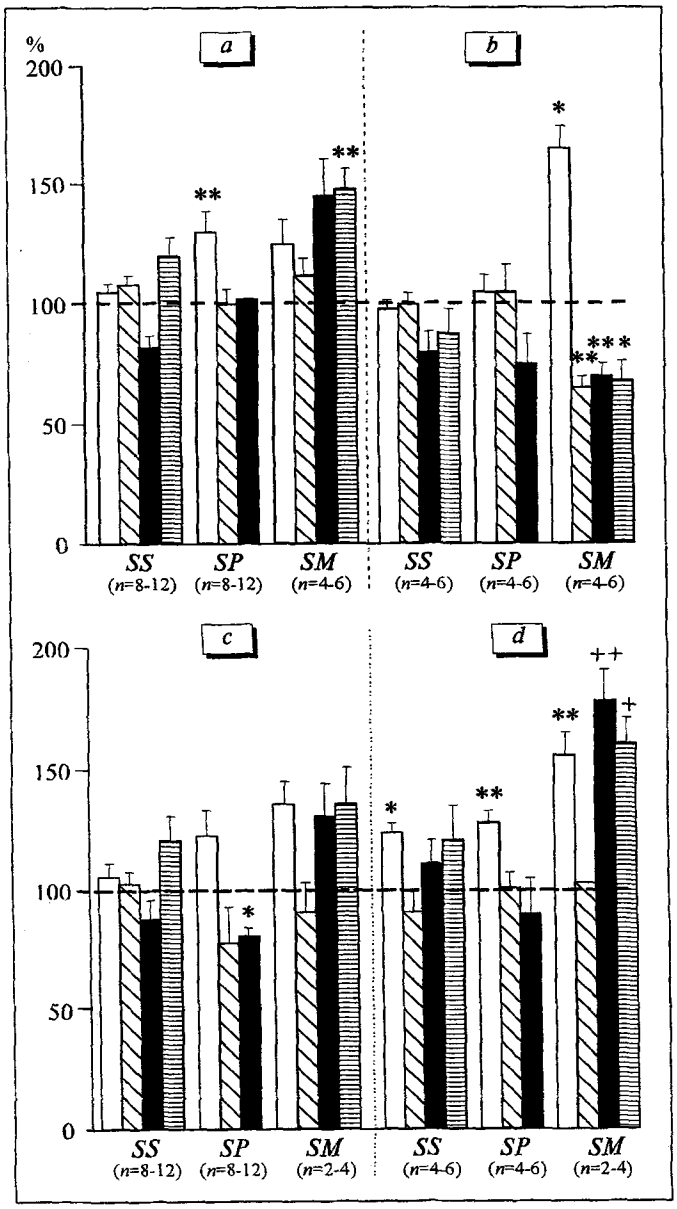

Fig. 1. Proportions between the contents of protein (*1*), choline acetyltransferase (2), acetylcholinesterase (3), and Na,K-ATPase (*4*) in fractions and subfractions of neocortical and hippocampal synaptosomes in intact rats with low and high resistance to hypoxia. Enzyme activities were measured per gram tissue. The values for low resistant rats are expressed as percent of values for high resistant rats. (Dashed line corresponds to 100%). Light (a) and heavy (b) synaptosomes in the neocortex; light (c) and heavy (*d*) synaptosomes in the hippocampus. Fractions: of synaptosomes (SS), of synaptoplasm (SP), subfraction of synaptic membranes (SM). Significantly different: *p=0.05, **p=0.025 (Fisher's *t* test); +$p<0.05$, ++$p<0.02$ (Student's *t* test).

RESULTS

Two groups of distinctions between the neocortex and hippocampal synaptic structure characteristics for LR and HR rats have been found. One group was typical of the neocortical D fraction and the other of neocortical C synaptosomes and D synaptosomes of the hippocampus. Activities of m-ChAT, m-AChE, and Na,K-ATPase in synaptolemmal subfraction of neocortical D synaptosomes in LR rats were low (60-66% of that in HR rats). By contrast, the portion of membrane proteins in LR rats was by 65% greater than in HR rats. The activity of c-AChE in the synaptoplasmic subfraction in LR rats was lower than that in HR rats. Both c-ChAT activity and the portion of water-soluble proteins were the same in LR and HR rats (Fig. 1, *b*). In fractions C of the neocortex and D of the hippocampus, m- and c-ChAT activities in LR and HR rats differed insignificantly, whereas m-AChE, Na,K-ATPase, and the portion of membrane and water-soluble proteins were greater in LR rats than in HR rats by 48-78, 49-57, 31-56, and 30%, respectively (Fig. 1, *a, d*). Similar, although statistically insignificant, differences were observed for C fraction of the hippocampus (Fig. 1, *c*). It should be noted that all the differences corresponded to the subsynaptic fractions (Fig. 1, *a-d*). This may result from greater structural and functional homogeneity of these fractions compared with those of synaptosomes.

Thus, the differences in m-ChAT activity between LR and HR rats were characteristic of neocortical D fraction. Any molecular form of ChAT is a marker enzyme of cholinergic neurons. Hence, fraction D of synaptosomes, for which the differences between the LR and HR rats were found, is cholinergic in nature. This is in consistency with the data on m-AChE activity, which positively correlates with m-ChAT activity (Fig. 2, *b*). No differences in ChAT activities were found for neocortical C and hippocampal D fractions. Hence, synaptosomes from these fractions were not cholinergic as to their mediator specificity.

The differences between such synaptosomes in the LR and HR rats may be explained either by different numbers of synaptosomes in the fractions or by their specific synaptic ultrastructure and molecular mechanisms. In this relation, the most indicative are comparative data on protein portion in subsynaptic fractions. In the former case, both membrane-associated and water-soluble protein portions in synaptosomes should be different. Indeed, such differences were found in neocortical C and hippocampal D fractions, where the coefficients of correlation between the contents of membrane-associated and water-soluble protein were, respectively, 0.67 (n=10, p<0.05) and 0.94 (n=6, p<0.05). One can conclude that the numbers of synaptosomes in these fractions were increased in proportion to the numbers of proper nerve terminals in the neocortex and hippocampus in LR rats. No correlation was found between membrane-associated and water-soluble protein portions in neocortical D fraction of LR and HR rats. In addition, the differences in membrane protein portions in LR rats did not correlate with the activity of membrane-associated enzymes. This implies different ultrastructural and molecular organisation of cholinergic synaptic membranes in neocortical D fraction in LR and HR rats. The portion of membrane proteins in this fraction did not correlate with the activity of any of the three membrane-associated enzymes, and, hence, represents an independent discrepancy characteristic of other, noncholinergic synapses of fraction D.

Low activity of m-ChAT and m-AChE in neocortical D fraction in LR rats may reflect inefficacy of proper cholinergic synaptic contacts, since it is m-AChE (extracellular form) that is responsible for acetylcholine catabolism from the synaptic cleft. The evidence on Na,K-ATPase activity, which positively correlates with those of m-ChAT and m-AChE, confirms this supposition (Fig. 2, *b*). Strong correlation among the three enzymes suggests that they belong to morphologically identical elements associated with cholinergic synapses of fraction D. Low activity of

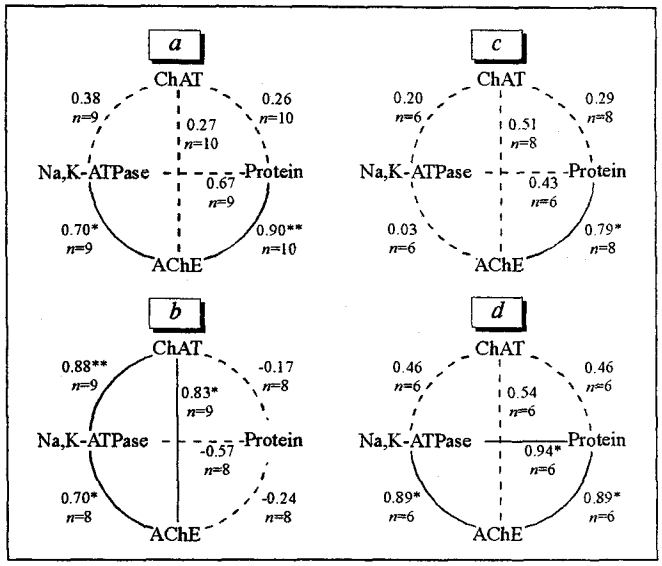

Fig. 2. Correlations between activities of membrane-associated forms of choline acetyltransferase (m-ChAT), acetylcholinesterase (m-AChE), Na,K-ATPase, and membrane protein content in synaptolemmal subfractions. Light (*a*) and heavy (*b*) synaptosomes in the neocortex; light (*c*) and heavy (*d*) synaptosomes in the hippocampus. The correlation coefficients are shown as numbers near lines connecting proper biochemical parameters; significance: *p<0.05, **p<0.01 (Spearman's rank correlation test).

Na,K-ATPase in neocortical D fraction leads to a longer period of ionic balance restoration, which is indicative of a week transmission efficacy in synapses. Furthermore, synaptic Na,K-ATPase is known to be functionally coupled with mediator reception. This enzyme and cholinoreceptor were shown to form a single molecular complex [2]. A little activity of Na,K-ATPase in the given situation may, therefore, be ascribed to low portion of cholinoreceptors in the membranes of LR rats.

In neocortical C and hippocampal D fractions the protein portion positively correlated with m-AChE and Na,K-ATPase activities (Fig. 2, *a*, *d*). This suggests that noncholinergic synapses, whose concentration is increased in the neocortex and hippocampus of LR rats, are AChE-reactive with a potent Na,K-pumping system and hence functionally highly effective. The presence in the central nervous system of AChE-positive noncholinergic neurons was confirmed by modern immunohystochemical methods [11]. In hippocampal C fraction no significant correlation was found between the test parameters (Fig. 2, *c*). So, the data obtained for this fraction may represent the total contribution of independent synaptic groups. However, it seems more likely that the same synaptic group (large enough) in both C and D hippocampal fraction is responsible for the differences between LR and HR rats. Located predominantly in fraction D, they provide the most significant differences in this fraction.

According to immunohystochemical evidence, there are two main origins of cholinergic nerve fibers in the neocortex (neocortical neurons and those of large cellular basal nucleus) [7], and one in the hippocampus (neurons of the forebrain basal nuclei — medial septal and diagonal band vertical nucleus) [11]. Both cholinergic systems are involved in vasodilatation, although having different humoral factors as a stimulus [6]. Synapses of neurons originating from the large cellular basal nucleus are predominantly accumulated in fraction C, while synapses of neocortical neurons in fraction D [3,4]. In our experiments, ChAT activities in C and D neocortical synaptosome fractions were, respectively, 15.72 and 2.68 nM Ach/g/min for LR rats, and 14.69 and 2.7 nM Ach/ g/ min for HR rats. Hence, in both groups, 85 % of total enzyme activity in neocortical synaptosomes fell on fraction C, and 15% on fraction D, which is similar to the proportion between the densities of ChAT-positive neurites in the large cellular basal nucleus and the neocortex (75 and 25%) [7]. This represents another argument in favour of our hypothesis. Thus, the results can be interpreted as follows. The efficacy of synaptic transmission in neocortical cholinergic neurons in LR rats is lower than in HR rats, and it is the same as concerns the two cholinergic systems of basal nuclei neurons innervating both the neocortex and hippocampus. However, an elevated concentration of noncholinergic but AChE-reactive synapses in the neocortex and hippocampus in LR rats indicates different distribution of cholinergic pathways in both cerebral formations in intact rats with low and high resistance to oxygen deficiency, that may play an important role in organisation of adaptation and/or pathological processes in response to hypoxic or ischemic stimulus.

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