EFFECT OF LIGHT DEPRIVATION ON BINDING ACTIVITY OF SEROTONIN WITH LIGHT AND HEAVY SYNAPTOSOMES FROM VARIOUS BRAIN FORMATIONS

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The degree of binding of serotonin (5-HT) with light and heavy synaptosomes in various brain formations of control and visually deprived rabbits was studied by the writer's gel-filtration method. The activity of this process in heavy synaptosomes of control rabbits was equal in the visual cortex and superior colliculus and significantly higher than in the motor cortex. Light synaptosomes of all structures tested were similar in their degree of 5-HT binding. During light deprivation the intensity of this process in the heavy synaptosomes in formations of the visual system fell by 74-81%, whereas in the same synaptosomes of the motor cortex it fell by 31%. The decrease in 5-HT binding in the light synaptosomes of all formations tested was equal, mainly by about 73% of normal. The results are discussed in the light of the possible mediator or modulating role of 5-HT in formations of the visual system.

KEY WORDS: *light deprivation; visual system; light and heavy synaptosomes; interaction between serotonin and receptor.*

Depriving developing animals of visual impulsation is known to cause a disturbance of their neurophysiological, conditioned-reflex, and behavioral responses [1]. These disturbances are unquestionably based upon morphochemical changes in the neurons and synaptic structures of the visual system [3]. The writer has shown [5, 15] that light deprivation inhibits the binding of serotonin (5-HT) and tryptamine by the total synaptosome fraction in individual brain formations. These changes in the formations of the visual system — the visual cortex and superior colliculus — were more marked than in other regions of the brain. Different types of synaptosomes (light and heavy) differ in the activity of various enzymes and they respond differently to deprivation of animals of visual impulsation [2]. Biochemical differences between the above-mentioned populations of synaptosomes have been reported in the brain of normal animals [8, 12].

The object of this investigation was to study 5-HT binding separately with subfractions of light and heavy synaptosomes in different parts of the brain of visually deprived animals. Interest in this problem is also determined by the fact that a mediator and modulating role of 5-HT in the functioning of the visual system of the brain has been postulated [5, 14].

EXPERIMENTAL METHOD

Experiments were carried out on two groups of rabbits: 1) animals aged 2.5 months (control), and 2) animals kept from birth until the age of 2.5 months in total darkness. The rabbits of group 2 were decapitated in total darkness. All operations with the brain were carried out at 0°C. The unpurified mitochondrial fraction was isolated from homogenates of the visual cortex, superior colliculus, and motor cortex of the brain of the control and visually deprived rabbits by differential centrifugation; light and heavy synaptosomes were isolated from this fraction by centrifugation in a stepwise sucrose density gradient (1.4-0.8 M) [9]. The intensity of binding of 5-HT with synaptosomes (5-HT $5 \cdot 10^{-5}$ M; ethanolamine-HCl, pH 10.1, 0.1 M; KCl 0.1 M; sucrose 0.1 M; 0-4°C) was determined by the writer's method

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TABLE 1. Binding of Serotonin (in nmoles 5-HT/mg protein) with Light and Heavy Synaptosomes of Various Brain Formations of Control Rabbits (M ± m)

Brain formation	Synaptosomes	
	light	heavy
Visual cortex	23,56±2,86	28,05±1,11
Motor cortex	22,37±4,22	16,17±1,16*
Superior colliculus	24,80±2,79	27,13±2,09

*Differences significant ($P < 0.05$) relative to visual cortex and superior colliculus in heavy synaptosomes.

[4, 5] based on gel filtration. The quantity of 5-HT bound with synaptosomes was calculated by the equation of Fairclough et al. [10]. The protein content was determined by Lowry's method [11]. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Data showing the degree of binding of 5-HT with fractions of light and heavy synaptosomes of different brain formations of the control rabbits are given in Table 1. Interaction between the mediator and the serotonin receptor of the postsynaptic membranes was judged on the basis of this index. In the control animals light synaptosomes of structures of the visual system and motor cortex were similar as regards their intensity of 5-HT binding. In the case of heavy synaptosomes the binding was practically identical in the visual cortex and superior colliculus and significantly higher than in the motor cortex. Comparison showed that interaction between 5-HT and receptor was virtually the same in both types of synaptosomes (heavy and light) of the visual system. Meanwhile the heavy synaptosomes of the visual cortex showed a tendency to interact less intensively with 5-HT than the light synaptosomes.

In an earlier study of the total synaptosome fraction of rabbit brain 5-HT was found [5, 15] to bind with the synaptosomes of structures of the visual system more actively than with synaptosomes of the "remaining" cortex (motor cortex and part of the parietal cortex). In the present investigation this pattern was observed only with respect to the heavy synaptosomes (Table 1).

De Robertis [8] showed that 5-HT is concentrated mainly in the fraction of light synaptosomes. Our own observations show that the 5-HT content in the tissue of the visual cortex is about one-third of that in the superior colliculus [6]. In the present experiments 5-HT interacted virtually equally actively with light and heavy synaptosomes in both formations of the visual system. These results thus confirm the earlier hypothesis [6] that the general occurrence of reserves of 5-HT in nerve tissue is not the only criterion that the compound is a mediator. In particular, it has been shown [13] that in the limbic cortex, in which the 5-HT content is much lower than in the medulla, the maximal rate of reassimilation of 5-HT by the synaptosomes is higher than in the medulla.

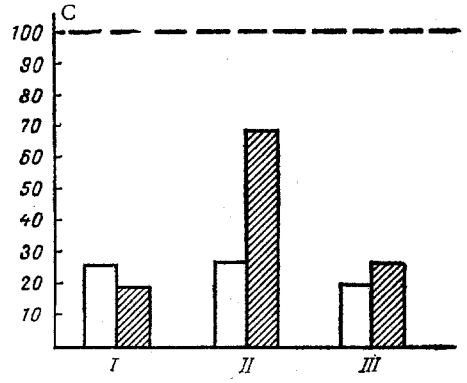

Fig. 1. Effect of light deprivation on degree of binding of 5-HT with light (unshaded columns) and heavy (shaded columns) synaptosomes of various formations of rabbit brain (in % of normal level). I, II, III) Visual cortex, motor cortex, and superior colliculus respectively. C) Control.

Analysis of the results in Table 1 suggests that receptors binding 5-HT are about equally numerous in the light synaptosomes of all the brain formations studied. Meanwhile, in the heavy synaptosomes of the visual cortex and superior colliculus these receptors are probably more numerous than in the heavy synaptosomes of the visual cortex.

During light deprivation a practically equal decrease in the degree of interaction between 5-HT and the light and heavy synaptosomes of the visual cortex and superior colliculus was observed (Fig. 1). This agrees with previous observations [5] made on the total synaptosome fraction. That investigation also showed that the binding of 5-HT with the total synaptosome fraction of the "remaining" cortex fell by a lesser degree than binding with synaptosomes in the formations of the visual system. The present experiments revealed differences in the activity of 5-HT binding only with respect to the heavy synaptosome fraction. Whereas in the visual cortex and superior colliculus the intensity of this process in the heavy synaptosomes fell by 81 and 74% respectively, in the corresponding synaptosomes of the motor cortex it fell by only 31% compared with normal (Fig. 1). In the light synaptosomes of all formations studied, the decrease in the degree of binding was virtually identical (by about 73%).

The results emphasized some differences between the heavy synaptosomes of formations of the visual system and those of the motor cortex: a higher intensity of binding with 5-HT under normal conditions and a greater decrease in its intensity during deprivation. This difference is probably due to the special role of 5-HT as a mediator or modulator in heavy synaptosomes of the structures of the visual system. Tebecis and Dimaria [14] found that 5-HT inhibits the activity of the relay neurons of the lateral geniculate body – a relay structure of the visual system. There are indications in the literature that the heavy synaptosomes are evidently derivatives of inhibitory synapses [8]. Our own observations show that visual deprivation affects not only inhibitory synapses (heavy synaptosomes), but also other types of synapses (light synaptosomes), reducing their degree of binding with 5-HT.

After light deprivation at an early age, the age differentiation of certain types of synaptic structures and of the receptor apparatus of neurons of the visual system of the animals' brain is thus evidently delayed. This hypothesis is confirmed by the results of morphological, physiological, and biochemical investigations [1-3, 7].

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