MONOAMINE OXIDASE ACTIVITY AND SEROTONIN METABOLISM IN THE RAT BRAIN DURING LATENT INHIBITION

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To investigate processes of selective attention in experiments on animals a model of latent inhibition (LI) is used. Latent inhibition is characterized by delay in the formation of a conditioned response to the frequent unreinforced presentation of a conditioned stimulus before its combination with the conditioned stimulus [8]. By the use of pharmacologic preparations specific for serotonin and the neurotoxin 5,7-dihydroxytryptamine in behavioral experiments, the important role of the serotoninergic system in the development of LI was demonstrated [3, 4, 12]. However, changes taking place in the brain at the neurochemical level and responsible for the LI process have not yet been adequately studied. In the investigation described below activity of monoamine oxidase (MAO), which is involved in serotinin metabolism and regulates its level and reuptake [7, 11], and also the concentrations of endogenous serotonin and 5-hydroxyindoleacetic acid (5-HIAA), formed as a result of oxidative deamination of serotonin by MAO, were determined in brain structures of rats with LI of a conditioned passive avoidance reaction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. A conditioned passive avoidance reaction (CPAR) was formed in the usual way [6] in a system consisting of a lit (safe) compartment and an unlit (punishable) compartment, in which the animals were subjected to a single electric shock (0.75 mA, 2 sec), and were quickly returned to their home cage. Preservation of the CPAR was tested after 1 day. The latent period (LP) of the change from the lit to the unlit compartment was recorded (time of observation 180 sec). Latent inhibition was produced in accordance with a special program [2] with long-term habituation to the conditioned stimulus (20 exposures in the experimental chamber) which preceded training. All the animals were divided into four groups: 1) intact control, 2) trained control with formation of a conditioned reaction but without preexposure to the conditioned stimulus, 3) with preexposure to the conditioned stimulus but without training, 4) with both preexposure and training (LI). The animals were decapitated immediately after preexposure or testing of the CPAR, the brain was removed and the frontal cortex, striatum, amygdaloid complex, and hippocampus were isolated in the cold. To determine MAO activity the tissue was homogenized in 0.32 M sucrose and the unpurified mitochondrial fraction (P_2) was isolated from the homogenates and MAO activity in it was determined by the method described previously [1]. Activity of the enzyme was measured in the presence of a saturated concentration of serotonin (3 mM). Protein in the homogenate was determined by Lowry's method. Serotonin-creatine sulfate ("Sigma") was used as the substrate. Serotonin and 5-HIAA were determined by a fluorometric method [5]. Probenecid ("Sigma"), used to block the active transport system for removal of 5-HIAA from the cell into the blood stream, was injected intraperitoneally in a dose of 200 mg/kg 2 h before testing (the time of maximal action of the preparation) [10]. The results were subjected to statistical analysis by Student's t test.

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TABLE 1. Mitochondrial MAO Activity (in nmoles ammonia/mg protein/min) and Serotonin and 5-HIAA Concentrations (in mg/g tissue) in Various Rat Brain Structures during Latent Inhibition (M ± m)

Groups	Amygdaloid complex			Striatum			Frontal cortex		
	MAO	serotonin	5-HIAA	MAO	serotonin	5-HIAA	MAO	serotonin	5-HIAA
1. Intact control	2.6±0.12 (8)	983±62 (6)	863±68 (6)	2.7±0.11 (11)	606±44 (8)	615±40 (8)	2.0±0.16 (10)	328±22 (7)	463±39 (7)
2. Trained control	3.4±0.20* (12)	952±53 (8)	547±30* (8)	1.8±0.07* (14)	548±39 (11)	474±18* (11)	2.0±0.19 (10)	313±20 (8)	408±13 (8)
3. Pre-exposure to conditioned stimulus	3.9±0.40* (9)	619±48* (6)	916±45 (6)	3.2±0.19* (9)	497±28 (7)	808±51* (7)	2.2±0.16 (9)	347±23 (7)	524±45 (7)
4. Pre-exposure + training (LI)	3.2±0.17* (6)	774±49** (6)	992±134** (6)	2.8±0.20** (13)	589±30 (7)	928±43** (7)	1.3±0.10** (8)	346±24 (6)	612±52** (6)

Legend. *$p < 0.05$ Compared with intact control, **$p < 0.05$ compared with trained control. Number of animals shown between parentheses.

EXPERIMENTAL RESULTS

Preexposure to the conditioned stimulus led to weakening of reproduction of CPAR (LP = 62.3 ± 18.3 sec) compared with the control group (LP = 149.6 ± 36.1 sec, $p < 0.05$), i.e., to the effect of LI (group 4). According to the data in Table 1, an increase in MAO activity with respect to serotonin deamination was observed in the animals of group 3 compared with group 1 in the amygdaloid complex (AC) and striatum. MAO activity in the frontal cortex was unchanged. With a combination of preexposure and conditioning (group 4) increased MAO activity in AC also was observed, whereas in the striatum MAO activity was unchanged compared with the intact control (group 1). In the frontal cortex MAO activity was reduced compared both with group 3 and with group 2.

Determination of the serotonin and 5-HIAA levels in the brain structures tested showed (Table 1) that the serotonin level in AC in the animals of group 3 was lower than in group 1, but the 5-HIAA level was unchanged. In LI (group 4) the lowered serotonin level continued and the 5-HIAA concentration was the same as in the animals of groups 1 and 3. A high 5-HIAA level was observed in the striatum of the rats of groups 3 and 4. In this case the serotonin level was unchanged. The same effect on the serotonin level also was observed in the frontal cortex.

The results thus demonstrate that changes in serotonin metabolism during LI take place even in the preexposure stage. Since the level of the metabolite in AC of the preexposed animals was unchanged by an increase in MAO activity, in order to discover whether the contradiction observed was associated with activation of 5-HIAA transport from the cell, we used the drug probenecid,which blocks active transport of organic acids in the brain [10]. Injection of probenecid into the preexposed animals significantly increased the 5-HIAA concentration in AC compared with the control (Fig. 1), evidence of enhancement of active 5-HIAA transport in group 3, and explaining the disparity found between enzyme activity and metabolite level. The fall of the serotonin level in animals with preexposure to the conditioned stimulus was more probably connected with activation of catabolism than with depression of its synthesis, for after injection of probenecid the serotonin level in this group of animals rose considerably. Thus reducing the significance of the stimulus at the preexposure stage is accompanied by enhancement of serotonin metabolism in AC. During testing of LI after conditioning of the preexposed stimulus, after administration of probenecid the levels of serotonin and its metabolite also were raised (Fig. 1), and in conjunction with data showing increased MAO activity, this indicates maintenance of a high level of serotonin metabolism in MC in the presence of LI also. Similar changes on preexposure of the conditioned stimulus were observed in the striatum. The increase in MAO activity, elevation of the 5-HIAA level, and absence of a fall in the serotonin level, despite high MAO activity, are evidence of activation of serotonin metabolism in the striatum also.

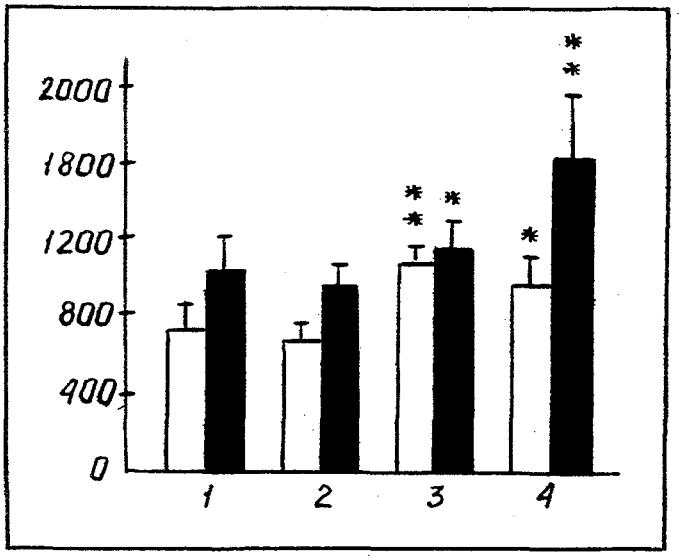

Fig. 1. Serotonin concentration (unshaded columns) and 5-HIAA (black columns) (in ng/g tissue) in the amygdaloid complex of the rat brain after administration of probenecid: 1) intact control, 2) trained control, 3) preexposure to conditioned stimulus, 4) preexposure + training (LI) *$p < 0.05$ Compared with corresponding control.

In the frontal cortex no change was observed either in the serotonin and 5-HIAA concentrations or in MAO activity on preexposure to the conditioned stimulus. On testing of LI, MAO activity fell, but the 5-HIAA level rose, possibly due to delay in removal of the metabolite from the cell.

No change in MAO activity was found in the hippocampus: in preexposed animals (group 3) it was 2.1 ± 0.16, compared with 2.2 ± 0.15 in the intact control; during LI MAO activity was 2.1 ± 0.32, compared with 2.2 ± 0.22 nmoles/mg·min in the trained control.

The results are thus evidence of the specificity of involvement of the serotoninergic system of different brain structures in the maintenance of the various stages of LI formation. Activation of metabolism takes place in AC and the striatum as early as the pre-exposure stage, and persists during LI. In the frontal cortex changes are observed only after conditioning of the preexposed stimulus during LI. An important role in the inhibition of attention to uninformative stimuli is ascribed to the hippocampus [9]. However, the absence of changes in serotonin deamination by MAO evidently indicates that the influence of the hippocampus on LI is effected through other neurotransmitter systems.

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