ROLE OF SEROTONIN RECEPTORS OF THE AMYGDALA AND CENTRAL GRAY MATTER IN CONDITIONED PASSIVE AVOIDANCE RECALL IN RATS

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Two main types of serotonin receptors, differing in their affinity for serotonin, are now distinguished in the brain (S_1 and S_2) [8, 11]. It was shown previously that an important role in the mechanisms regulating formation and recall of the memory trace is played by the amygdala (AM) and central gray matter (CGM) of the mesencephalon [1, 2, 14]. These structures have powerful afferent connections with the mesencephalic nuclei raphe [3, 4]. However, very little is known about the role of the serotoninergic system of AM and CGM in the processes of memory formation.

The aim of this investigation was to determine the role of C_1-receptors of AM, CGM, and the frontal cortex, which are the most sensitive to serotonin, in the processes of withdrawl of the memory trace. The conditioned passive avoidance reflex (CPAR) was used as correlate of the memory trace.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The CPAR was formed [6] on the basis of a single electrodermal reinforcement (current 1 mA, 6 sec) in an apparatus consisting of two compartments – one lit (safe), the other dark (unsafe). The latent period (LP) of changing from the lit to the dark compartment was recorded. The CPAR was tested 24 h after its formation by placing the animal in the lit compartment. The conditioned reflex was considered to have been formed if LP of the switch of compartment was not less than 180 sec. Rats whose LP of the switch of compartment was between 2 and 30 sec were regarded as untrained. Rats also were placed in the chamber but did not receive electrodermal stimulation served as the control. Some of the animals taught CPAR were decapitated after 24 h without testing, their brains were removed and, using the atlas [7], AM, CGM, and the frontal cortex were removed in the cold. Specific binding of S_1-receptors with serotonin was determined by the radioligand method [11] in the membrane fraction of homogenates of the corresponding brain structures. The tissue was homogenized in 50 mM Tris-HCl buffer, pH 7.4, and centrifuged at 20,000g for 20 min to sediment the membrane fraction. The residue was homogenized in the same buffer and incubated for 10 min at 37°C to remove endogenous serotonin, and then centrifuged again for 20 min at 20,000 g. ^{3}H-serotonin (21.8 Ci/mmole, from "Amersham") was used as the radioligand. Incubation of the samples containing 0.5 ml of tissue suspension, corresponding to 15 mg of the original wet weight of the tissue, 0.1 ml of ^{3}H-serotonin, 0.5 ml of unlabeled serotonin ("Reanal"), and 1 ml buffer, was carried out for 10 min at 37°C in 50 mM Tris-HCl, pH 7.4, containing 4 mM $CaCl_2$, 5.7 mM ascorbic acid, and 10 μm pargyline. Incubation was stopped by rapid cooling in ice-cold water followed by centrifugation. The residues were dried and covered with scintillation fluid. Bound radioactivity was measured on a "Delta-300" liquid scintillation counter with 55% efficiency. Specific binding of the labeled ligand with the receptors was calculated as the difference between its binding in the absence (total binding) and presence of 1 uM unlabeled serotonin (nonspecific binding), and expressed in fmoles/mg protein. Protein in the samples was determined by Lowry's method [9]. The kinetic parameters, namely K_d and the maximal number of binding sites, were determined in the presence of 17 concentrations of ^{3}H-serotonin in the range from 0.5 to 42 nM. The experimental data were subjected to computer analysis by means of one- and two-site binding models [5]. The criterion of choice between one- and two-site binding models was the sum of the squares of the deviations for these models (F criterion). The results were analyzed by Student's t test.

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TABLE 1. Specific Binding of ^{3}H-Serotonin (2.5 nM) with Membranes of AM, CGM, and Frontal Cortex (in fmoles/mg protein) in Control Rats and Rats Trained in CPAR (M ± m)

Group of animals	Amygdala	Central gray matter	Frontal cortex
1 – control	67,1±3,2 (16)	85,1±4,3 (11)	57,9±2,8 (10)
2 – electrodermal stimulation	75,6±7,8 (6)	84,2±6,9 (3)	47,8±5,6 (8)
3 – trained in CPAR and tested	48,9±3,4* (16)	58,7±4,7* (6)	55,8±2,1 (9)
4 – trained in CPAR without testing	65,2±5,0 (10)	85,6±5,1 (4)	49,7±4,2 (8)
5 – not trained in CPAR and tested	76,3±3,3 (6)	77,8±5,6 (4)	59,8±5,7 (7)

Legend. *$p < 0.01$ compared with control; number of experiments shown in parentheses.

EXPERIMENTAL RESULTS

During CPAR changes were found to take place in receptor binding of serotonin. In trained rats found to preserve the reflex when tested 24 h later (group 3) specific binding of serotonin was reduced (Table 1). These changes were found only in AM and CGM, but in the frontal cortex serotonin binding did not differ from the control level in any of the groups of animals tested, evidence of unequal involvement of S_1-receptors of different serotoninergic brain stuctures in CPAR. The training procedure itself did not affect the level of serotonin binding: in animals killed immediately after electrodermal stimulation (group 2) no difference from the control was found.

To determine at what stage of training the S_1 receptors of AM and CGM are involved in the mechanisms of memory regulation, serotonin binding was determined in rats subjected to the conditioning procedure but which, unlike the rats of group 3, were not tested, and therefore had no opportunity to recall the memory trace. Specific binding of serotonin in the animals of this group (four) was unchanged both in AM and in CGM compared with the control and it differed significantly from serotonin binding in group 3 ($p < 0.02$), indicating that S_1-receptors of AM and CGM are more likely to be involved in the processes of recall of the memory trace than of its formation. The above argument is confirmed by measurements of specific serotonin binding in trained, but not the untrained rats (group 5). These animals, when tested, did not reproduce CPAR and serotonin binding in them was the same as in the control. Analysis of these data reveals changes in S_1-receptor binding only in animals which reproduced the conditioned reflex.

Determination of the kinetic parameters of serotonin binding (K_d and the maximal number of receptors – B_{max}) in AM of the control animals showed that the experimental data are better approximated by the two-site model of binding (Fig. 1), with $K_d = 0.50 \pm 0.05$ nM and with $B_{max} = 34.0 \pm 3.5$ fmoles/mg protein for sites with high affinity for serotonin, and with $K_d = 17.0 \pm 1.0$ nM and $B_{max} = 267 \pm 15$ fmoles/mg protein for sites with low affinity. In the trained animals, by contrast with the controls, a linear Scatchard plot was observed, evidence of one type of binding sites for serotonin with $K_d = 9.4 \pm 0.4$ nM and $B_{max} = 268 \pm 19$ fmoles/mg protein. Thus reduction of the functional activity of the serotinin receptors in AM is evidently linked with predominance of low-affinity binding in the trained animals.

Activity of the S_1-receptors showed a decrease either during retrieval of the memory trace or immediately thereafter. It acts as the background against which the process of trace retrieval develops, for without actual retrieval of the memory trace, even if the conditioned stimulus appeared, no decrease of activity takes place.

We know from data in the literature that the serotoninergic system can have an inhibitory effect on AM [12, 13, 15]. It has been claimed that the inhibitory action of serotonin in

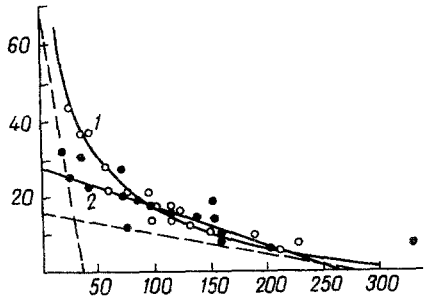

Fig. 1. Kinetics of binding of ^{3}H-serotonin with membrane fraction of AM in control and conditioned rats (Scatchard plot). Abscissa, quality of bound ligand (in fmoles/mg protein); ordinate, ratio of bound to free ligand. Each point is mean value of four experiments. Theoretical curve for control animals – 1) and straight line (for rats taught CPAR – 2) shown on graph. Broken lines denote asymptotes (linear regions for two-site model of binding).

the CNS is mediated through S_1-receptors [10]. It can be tentatively suggested that reduction of the functional activity of S_1-receptors in the present experiments is accompanied by activation of neurons in AM and CGM which are components of the emotiogenic regulatory system of memory, and it is connected with the emotional-autonomic components of the memory trace.

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