Effect of Active Immunization with Plasma Aminooxidase on Mnestic Processes and Transmittory Systems in Rat Brain

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Immunization to bovine plasma amine oxidase improves conditioned reactions in rats. This effect is accompanied by inhibition of plasma amine oxidase and monoamine oxidase A, a decrease in the content of 5'-hydroxyindolacetic acid, and accumulation of serotonin, dopamine, and norepinephrine in neuronal mitochondria of the sensorimotor cortex and caudate nucleus.

Key Words: *benzylamine oxidase; immunization; monoamine oxidases A and B; active avoidance*

The functions of mitochondrial monoamine oxidases (MAO), important regulators of monoamine concentration in the nervous system, are studied in detail. There is special amine oxidase in the blood plasma, which is assigned to different groups of amine oxidases. Some investigators classify this enzyme as plasma amine oxidase, thus emphasizing its specificity [9], while others assign it to the MAO subtype and name it benzylamine oxidase (standard test substrate) [1,7,8]. This enzyme (hereafter referred to as PAO) is believed to regulate the proportion between different biogenic monoamines in the blood; however, its exact physiological role remains obscure. In view of this in the present study we used the method of active immunization to bovine PAO for long-term modulation of enzyme activity. We choose admittedly gentle immunization regimen to avoid complete elimination of PAO, i.e., true autoimmune disease. Structural similarity of analogous mammalian enzymes and, simultaneously, some peculiarities in their structure result in the formation of both antibodies against heterologous proteins and autoantibodies to own enzymes [11]. These autoantibodies inactivate and/or eliminate PAO by forming stable complexes with the enzyme. These results were obtained in our previous experiments.

We assume that autoantibodies against PAO can cross the blood-brain barrier, enter neurons and interact with mitochondrial MAO A and B. According to current views this process does occur in long-term circulation of the antibodies [6]. However, the similarity of mitochondrial MAO and PAO has not been studied yet.

In the present study we examine the effect of active immunization to PAO on mnestic functions, in particular, conditioning of active avoidance reaction (AAR), based on learning of different combinations of conditioned and unconditioned stimuli. In experimental animals, plasma activity of benzylamine oxidase and MAO activity and the content of biogenic amines in the brain were measured. The possibility of both direct and indirect effects of immunization against PAO on the studied parameters was taken into account.

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TABLE 1. Amine Oxidase Activity of Rat Plasma

Parameter	Control (physiological saline)	Rats immunized to plasma MAO	% of control (100%)
Specific activity, cpm/min/mg	909	663	73
	708	516	72.9
	1259	603	47.9
	1153	967	83.9
	1005	604	60
Median	967	604	62.4*

Note. *$p<0.05$ compared with the control (Wilcoxon test).

MATERIALS AND METHODS

Experiments were carried out on male albino rats (Kryukovo nursery) weighing 180-200 g. The animals were immunized with PAO (MAO) from bovine plasma with activity of 0.002 arb. units/mg (Sigma). To this end, experimental animals were injected with MAO (40 μg per rat) without adjuvant into the caudal vein. Control animals received physiological saline. One month later, the experimental rats were subcutaneously injected with 0.4 ml mixture containing MAO and complete Freund's adjuvant (1:1, 800 μg/kg) subcutaneously into four sites of the back. Control animals were divided into 2 groups: group 1 animals received complete Freund's adjuvant with physiological saline, while group 2 received saline only. Learning of AAR in a shuttle box was started 20 days after the second injection.

The active avoidance reaction was conditioned for 21 days in an automated shuttle box: the animals were presented 20 combinations of conditioned and unconditioned stimuli per day. The number of avoidances (to auditory stimulus), escapes (to electric shock), escape failures, and interstimulus reactions, as well as response latencies were assessed; 80% in experimental group served as the learning criterion. After the end of experiment, the titer of anti-MAO antibodies was measured by enzyme-linked immunosorbent assay. To evaluate the retention of AAR and the dynamics of conditioning, a 10-day interval was made between days 7 and 17.

Activity of PAO was measured by radioligand assay [5]: plasma samples (up to 6 mg protein per sample) were incubated at 37°C for 1 h with 4.7 μM ^{14}C-benzylamine (specific activity 0.3 Ci/mol, Amersham) in 0.08 M K,Na-phosphate buffered saline (pH 7.2). To this end heparinized blood from 5 immunized rats and 5 control animals was collected and the plasma was isolated by centrifugation at 7500 rpm and 0°C for 40 min.

After the end of conditioning, the activities of MAO A and B and the content of biogenic amines in the sensorimotor cortex and caudate nucleus of control and experimental animals were determined. The mitochondria were isolated by differential centrifugation. Protein content was measured by the method by Lowry; the activity of MAO A and was assayed using serotonin [10] and p-nitrophenylethylamine [2], respectively. The content of biogenic amines and 5'-hydroxyindolacetic acid in homogenates was measured fluorimetrically [3].

The data were processed statistically using nonparametric Wilcoxon and van der Varden tests (Stadia software).

RESULTS

The titer of anti-MAO antibodies in experimental animals on day 42 after the second injection of the antigen was 3200, while in two control groups this parameter was 225 and 125 respectively. This suggests the efficiency of the used immunization scheme.

TABLE 2. MAO A and B Activities in Different Brain Areas in Rats Immunized to PAO (% of Control)

Group	Sensorimotor cortex		Caudate nucleus	
	MAO A	MAO B	MAO A	MAO B
Complete Freund's adjuvant	45**	93	47**	86
Physiological saline	42**	119	35**	114

Note. Here and in Table 3: *$p<0.05$, **$p<0.001$ compared with the control (100%).

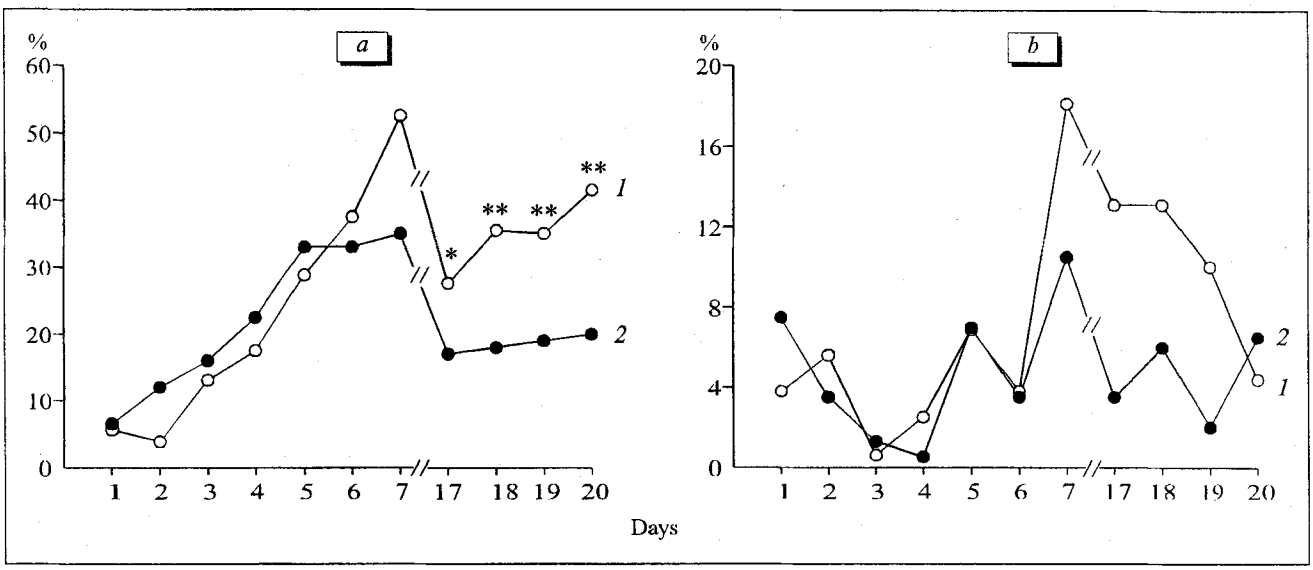

Fig. 1. Conditioning of active avoidance reaction in rats immunized to plasma amine oxidase. *a*) avoidance (to acoustic stimulus); *b*) number of interstimulus responses. *1*) plasma amine oxidase+Freund's adjuvant, 2) physiological saline+Freund's adjuvant. *$p<0.05$, **$p<0.001$ compared with 2.

Injection of complete Freund's adjuvant reduces pain sensitivity and other parameters in animals [4]; therefore, animals receiving adjuvant-saline mixture were used as the control in the analysis of behavioral data.

The amine oxidase activity in the plasma is not affected by the specific MAO inhibitor deprenyl (96% activity in the presence of 0.1 μM deprenyl) but is sensitive to the copper-containing oxidase inhibitor semicarbazide (63% activity in the presence of semicarbazide). Hence, this activity is due to the presence of classic plasma benzylamine oxidase (Table 1).

There were no considerable differences in the dynamics of AAR conditioning between the control and experimental groups until day 6 of learning (Fig. 1); on days 6-7, the number of positive responses was slightly increased in experimental group compared with the control. The effect of immunization was most pronounced upon retrieval of AAR after a 10-day interval (on day 17 of learning and day 32 after the second immunization). On days 17-19 ($p<0.05$) and 20 ($p<0.001$), the number of positive reactions in the experimental group considerably surpassed the control. Immunization also increased the number of interstimulus reactions, which attests to high generalization of conditioned reaction in the central nervous system.

After the end of AAR conditioning, we observed a considerable decrease in MAO A activity in the brain of experimental animals (Table 2); simultaneously, the content of serotonin in the caudate nucleus markedly increased, while the content of its metabolite 5'-hydroxyindolacetic acid significantly decreased (Table 3). The activity of MAO B remained unchanged, but the content of dopamine and norepinephrine in these brain areas increased.

Thus, immunization to bovine plasma PAO improved the retention of conditioned reactions, which manifested itself in a higher avoidance score (to acoustic stimulation) in the AAR. test on days 17-20 of learning. This effects was accompanied by inhibition of MAO A and a decrease in the content of the end-product of serotonin metabolism 5'-hydroxyindolacetic acid in the sensorimotor cortex and striatum, the content of serotonin in the striatum being 5.7-fold higher compared with the control. The content of dopamine and norepinephrine was also increased, although to a lesser extent.

TABLE 3. Content of Biogenic Amines in Sensorimotor Cortex and Caudate Nucleus of Rats Immunized to PAO (% of Control)

Biogenic amines	Sensorimotor cortex	Caudate nucleus
Dopamine	107	120*
Norepimephrine	125*	160**
Serotonin	83*	570**
5'-hydroxyindolacetic acid	48**	30**

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