EFFECT OF PROLONGED EXPOSURE TO COLD ON MONOAMINE OXIDASE ACTIVITY AND KINETICS AND SEROTONIN METABOLISM IN THE RAT BRAIN

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During prolonged exposure of warm-blooded animals to cold a specific combination of reactions develops and is aimed at ensuring constancy of the body temperature and increased resistance of the animal to low temperatures [5, 7]. Peripheral adaptive changes have received the most study whereas central mechanisms of transition of the temperature-regulating system to a new level of function during exposure to cold have received insufficient study [5]. The serotoninergic system of the brain plays an important role in the regulation of body temperature [8]. It has been shown that exposure to cold affects the serotonin level [10] and monoamine oxidase (MAO) activity in the brain [2], although the mechanisms of these changes is not yet clear.

In the investigation described below a combined study was made of the catalytic properties of MAO (amine: oxygen oxidoreductase, deaminating, flavine-containing, EC 1.4.3.4) and of serotonin metabolism during acclimatization of rats to cold.

EXPERIMENTAL METHODS

Male Wistar rats weighing 180-230 g were investigated. To study the dynamics of adaptive changes, tests were carried out after the animals had been kept for 1, 3, 7, and 14 days and 6 weeks in a chamber with an air temperature of 4-6°C. Rats kept in the animal house where the air temperature was 19-22°C served as the control. By differential centrifugation, an unpurified mitochondrial fraction [14] was obtained from a homogenate of the brain stem of decapitated rats, and MAO activity in it was determined by the method of Gorkin et al. [1]. Samples were incubated in 0.1 M K,Na-phosphate buffer, pH 7.4, at 37°C for 30 min in air. Activity of the enzyme was expressed in nanomoles of ammonia liberated during deamination of the substrate per minute per milligram protein. The protein concentration in the homogenate was determined by Lowry's method. To calculate kinetic parameters of the oxidative deamination reaction of serotonin — the Michaelis constant (K_m) and maximal reaction velocity (V_{max}) — the initial reaction velocities were measured with six different concentrations of serotonin (serotonin creatinine sulfate, from Reanal, Hungary) from 0.05 to 1 mM. The apparent K_m and V_{max} were found by a statistical method of analysis [3]. Besides kinetic studies of deamination of serotonin, the specific substrate for type A MAO [11], activity of type B MAO was determined for deamination of benzylamine (benzylamine·HCl) [15], which was used in a concentration of 1 mM. Serotonin and 5-hydroxyindoleacetic acid (5-HIAA) in the brain were determined by a fluorometric method [9]. Probenecid (from Serva, West Germany) was injected intraperitoneally in a dose of 200 mg/kg and the rats were killed 2 h after the injection.

EXPERIMENTAL RESULTS

After exposure of the rats to cold for only 1 day a decrease was observed in brain mitochondrial MAO activity with respect to serotonin deamination. The decrease was greater (by 23-28%) in the region of substrate concentrations close to K_m, i.e., in the region characteristic of physiological substrate concentrations [6]. With an increase in the serotonin concentration, in the incubation sample, the fall in enzyme activity became less marked (Table

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TABLE 1. Brain Mitochondrial MAO Activity (in nanomoles ammonia/min/mg protein) and Kinetic Parameters K_m (in mM) and V_{max} (in nmoles/min/mg protein) of Oxidative Deamination Reaction of Serotonin in Rats at Different Times of Acclimatization to Cold (M ± m)

Substrate	Control	Period of acclimatization, days				
		1	3	7	14	42
Serotonin, mM						
0,050	1,3±0,09 (15)	1,0±0,14 (9)	1,3±0,10 (8)	1,2±0,11 (12)	1,3±0,14 (8)	1,2±0,08 (13)
0,075	1,6±0,07 (17)	1,2±0,11 (7)**	1,4±0,15 (8)	1,4±0,13 (14)	1,7±0,12 (7)	1,6±0,07 (18)
0,100	2,5±0,15 (19)	1,8±0,14 (7)**	1,8±0,10 (15)***	1,5±0,10 (15)***	1,8±0,20 (7)*	1,8±0,11 (16)***
0,250	3,4±0,14 (18)	2,8±0,26 (8)	2,7±0,11 (9)***	2,9±0,14 (14)*	2,8±0,17 (8)*	2,7±0,13 (18)***
0,500	4,3±0,19 (17)	3,6±0,24 (8)	3,8±0,16 (9)	3,8±0,26 (13)	4,0±0,23 (8)	3,7±0,19 (18)*
1,000	4,9±0,25 (18)	4,2±0,35 (8)	4,6±0,20 (9)	4,8±0,28 (14)	4,9±0,24 (8)	4,7±0,20 (18)
K_m	0,164±0,019	0,209±0,031	0,164±0,039	0,209±0,032	0,170±0,043	0,172±0,014
V_{max}	5,8±0,36	5,1±0,44	5,0±0,63	5,5±0,48	5,4±0,73	5,1±0,22
3,000	5,8±0,17 (18)	6,2±0,22 (13)	5,0±0,20 (10)*			4,4±0,24 (18)***
Benzylamine (1 mM)	1,9±0,08 (15)	1,8±0,07 (13)	2,1±0,19 (10)			1,9±0,19 (11)

Legend. Number of experiments shown in parentheses. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

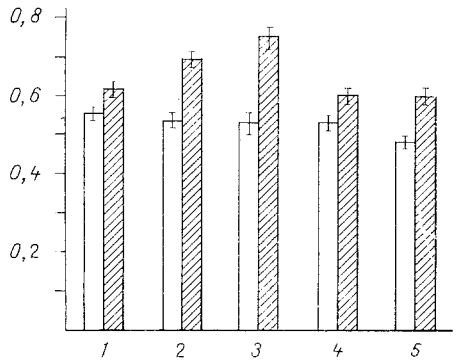

Fig. 1. Serotonin (unshaded columns) and 5-HIAA (shaded columns) concentrations in brain stem of rats during long exposure to cold. Ordinate, serotonin and 5-HIAA concentration (in µg/g tissue). 1) Control, 2) 1 day, 3) 3 days, 4) 14 days, 5) 6 weeks after beginning of exposure to cold.

1). A similar response of the monoamine oxidase enzyme system of the brain to exposure to cold for 1 day also was observed in the winter period. In summer no changes were found in enzyme activity, evidently because of differences in seasonal reactivity of the animal. After exposure to cold for 3 days MAO activity still remained low, in the region of K_m or above, but unlike the first day, a fall in MAO activity (by 14%) also was observed in the presence of a high concentration of substrate, saturating the enzyme (3 mM), at which the maximal reaction velocity was practically reached (Table 1). The decrease in MAO activity in the K_m region still remained after exposure to cold for 7 and 14 days. In animals acclimatized to cold MAO activity in the K_m region was 28-24% lower, and in the presence of serotonin in a concentration of 3 mM it was 24% lower (Table 1). The saturated enzyme is known to be unable to intensify its catalytic activity with a further increase in substrate concentration [3]. Saturation of the enzyme with substrate in acclimatized rats took place at a lower serotonin concentration than in intact animals. Whereas in the control MAO activity was lower with serotonin in a concentration of 1 mM than of 3 mM ($P < 0.001$), and it was below V_{max} ($P < 0.05$), in acclimatized rats activity of the enzyme with a substrate concentration of 1 mM

was indistinguishable from that in a concentration of 3 mM and at V_{max}, whereas MAO activity with serotonin in a concentration of 3 mM was actually below V_{max} ($P < 0.05$), evidence of substrate inhibition. K_m did not change significantly during acclimatization to cold and a small increase was observed after 1 and 7 days. The parameter V_{max} showed a tendency to decrease (Table 1). The results are evidence of structural changes in the monoamine oxidase enzyme system of the rat brain, accompanied by a fall in the velocity of catalytic deamination of serotonin in the region of physiological concentrations during prolonged exposure to cold, evidently as the result of conformational changes in enzyme structure.

No difference from the control level was observed in benzylamine deamination both during the first day of exposure to cold and after 6 weeks (Table 1). Type A MAO is evidently more sensitive to the action of cold than type B MAO.

Determination of endogenous concentrations of serotonin and 5-HIAA, the principal product of serotonin catabolism by MAO, showed that during the first 3 days of exposure to cold the serotonin level in the brain stem of the rats was unchanged, but the 5-HIAA level was raised. After 14 days the 5-HIAA and serotonin levels were indistinguishable from initially (Fig. 1). Since type A MAO activity during the first 3 days of exposure to cold was not increased (Table 1), it was suggested that the increase in the 5-HIAA level observed was the result of disturbances caused by the stress action of cold in the membrane system for active transport of the acid. Administration of probenecid, which blocks active transport of organic acids from the brain [13] confirmed this hypothesis. Probenecid raised the metabolic level in the brain, but no difference was found in the serotonin and 5-HIAA concentrations in the control rats and rats exposed to cold and treated with probenecid. After injection of probenecid the 5-HIAA level in the control animals was 1.29 ± 0.080 μg/g, during exposure to cold it was 1.29 ± 0.069 μg/g, and the corresponding serotonin concentrations were 0.68 ± 0.061 and 0.67 ± 0.054 μg/g tissue.

In animals acclimatized to cold the serotonin level was lowered by 13% ($P < 0.002$, Fig. 1). The fall in the brain serotonin level, with no accompanying increase in MAO activity, must be taken to indicate a fall in activity of the serotonin-synthesizing system of the rats' brain.

The results thus indicate modifications to activity of the enzyme systems of brain serotoninergic structures accompanied by a decrease in the intensity of serotonin metabolism during acclimatization of rats to cold. The fall in activity of the brain serotoninergic system during exposure to cold may perhaps be adaptive in character and linked with the inhibitory effect of serotonin on chemical heat production [12] and contractile thermogenesis [4].

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