Effect of the Catalase Inhibitor 3-Amino-1,2,4-Triazole on Oxidation-Phosphorylation Coupling and the State of the Mitochondrial Adenosine System in the Liver of Albino Rats

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Incubation of the specific catalase inhibitor 3-amino-1,2,4-triazole with liver mitochondria and administration of this drug to intact rats are shown to uncouple oxidation and phosphorylation and to inhibit adenosine nucleotide synthesis in the animal liver. These disturbances apparently result from catalase inhibition.

Key Words: *catalase inhibitors; liver; oxidative phosphorylation*

Previous studies have demonstrated the participation of catalase in the energy metabolism of the animal organism [1,3,7,8,12]. We observed this phenomenon in experiments with cytochrome *c*-deficient liver mitochondria. For instance, the addition of exogenous catalase to these mitochondria greatly stimulated phosphorylation processes [5,6,12].

In the present study we investigated the *in vivo* and *in vitro* effect of the specific catalase inhibitor 3-amino-1,2,4-triazole (AT), on oxidation-phosphorylation coupling and the state of the adenosine system.

MATERIALS AND METHODS

Mitochondria were isolated from the liver by differential centrifugation in a medium containing 0.3 M sucrose, 0.02 M Tris-HCl, and 0.001 M EDTA, pH 7.4. Oxygen uptake and the degree of coupling in the mitochondrial preparations were evaluated by the polarographic method using a Clark platinum electrode in a medium containing 0.25 M sucrose, 0.015 M KCl, 0.02 M KH_2PO_4, and 0.007 M $MgCl_2$, pH 7.4. The volume of the measuring cell was 1.5 ml. An aliquot of the mitochondrial suspension contained 4 mg protein. The protein concentration was measured after Lowry in the Agstein modification. For quantitative assay of adenosine nucleotides the liver was removed under light ether narcosis, frozen in liquid nitrogen, weighed, and reduced to powder in a porcelain mortar, after which an equal volume of 10% trichloroacetic acid in acetone was added to the powder and the homogenate was transferred to an ice-bound porcelain bowl. Ten percent aqueous solution of trichloroacetic acid was added to the homogenate in a ratio of 1:2. After the acetone had evaporated, the mixture was transferred to cooled test tubes and centrifuged at 12,000 g for 20 min in the cold. The supernatant was washed twice with cold sulfuric ether and used for nucleotide assay. Adenosine nucleotides were separated by high-voltage electrophoresis using a zinc-citrate buffer (pH 3.8) containing 0.035 M citric acid, 0.0148 M sodium citrate, and 0.003 M zinc acetate. The separation was carried out at 1000 V for 1.5-2 h on fast-separating chromatographic paper previously washed for 24 hours with 1.5-1.8% HCl. The electrophoretic pattern was visualized on an ultrachemiscope designed

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by E. M. Brumberg. The spots were cut off and eluted with 0.1 M HCl for 24 h at 18-20°C. The concentration of nucleotides in the eluates was determined spectrophotometrically at 260 and 290 nm and calculated by the formula: $[\Delta E \times 0.363 \times K_1]/K_2 \times K_3 = 1$ μmol/g tissue, where ΔE is the difference in extinction at 260 and 290 nm, 0.363 is the molar extinction coefficient for adenosine nucleotides, K_1 is the volume of the extract after centrifugation of the homogenates (in ml), K_2 is the volume of the extract loaded for separation (ml), and K_3 is the weight of the tissue sample (g).

Catalase activity (E) was measured after Brestkin—Novikova and calculated by the number of μmol of cleaved H_2O_2 per mg protein.

RESULTS

The catalase inhibitor AT (20 mg) added to the liver mitochondria suspended in the incubation medium in the presence of either NADH-independent (succinate) or NADH-dependent (glutamate+malate) substrates inhibits respiration, especially in the presence of a phosphate acceptor, and does not lower the rate of dinitrophenol-uncoupled respiration. A marked inhibition of phosphorylation and suppression of respiration control are also observed.

The changes in the parameters of tissue respiration were identical in the presence of both NADH-independent and NADH-dependent substrates. Low AT concentrations (below 20 mg) produced a less pronounced inhibitory effect, while higher concentrations greatly lowered the rate of respiration. Especially interesting is the fact that AT inhibits respiration after the ADP pool is depleted (state IV after Chance), since according to previous data [2] mitochondrial respiration in the controlled state may be considered to be physiological rather than activated.

In the next experimental series AT (100 mg/kg in sterile physiological solution) was intraperitoneally injected to random-bred albino rats weighing 180-200 g. The animals were sacrificed 1.5 hours postinjection and the parameters of respiration of liver mitochondria, the amount of adenosine nucleotides, and catalase activity were assayed. The dynamics of respiration in the liver mitochondria from the AT-treated animals was similar to that observed in *in vitro* experiments: a sharp inhibition of phosphorylation and no changes in the rate of dinitrophenol-uncoupled respiration. The disturbances in the oxidation-phosphorylation coupling in liver mitochondria induced by the intraperitoneal injection of AT are also confirmed by the experiments measuring the amount of adenosine nucleotides in liver tissues.

TABLE 1. Effect of AT on the Content of Adenosine Nucleotides in Rat Liver (Data from 20 Experiments)

Parameter	Amount of adenosine nucleotides, mmol/g tissue±12%	
	control	experiment
ATP	0.591	0.245
ADP	0.303	1.009
AMP	1.680	0.589
Energy. C	2.577	1.843

Intraperitoneal injection of AT was found to sharply reduce both the ATP content (to 41.5%) and the exchange pool of adenosine nucleotides (by 32.9-35%) (Table 1).

These findings together with the data on the state of oxidation-phosphorylation coupling clearly suggest that catalase inhibition impairs tissue respiration. The fact that AT inhibits catalase activity was proved by direct measurements in mitochondrial preparations and liver homogenates 1.5 hours after AT injection.

The data in Table 2 show that catalase activity in both mitochondria and liver homogenates is greatly diminished.

Previously we reported that catalase may be involved in coupled oxidative phosphorylation. An important role here is known to be played by flavoproteins possessing peroxidase activity. Consequently, peroxides, in particular H_2O_2, may arise in the tissue respiration chain [13]. We believe that H_2O_2 is instrumental in coupled oxidative phosphorylation: when attacked by catalase, it gives rise to a number of active radicals (OH, H, HO_2, etc.). The energy of these radicals goes for dehydrating the phosphate residues of adenosine nucleotides (ADP) and phosphoric acid. Recombination of these dehydrated phosphate residues results in the formation of high-energy bonds. Analogous conclusions have been reached by other authorities [9-11,14,15]. The experimental data presented here bolster our assumption on the role of catalase in tissue respiration.

TABLE 2. Catalase Activity in Mitochondria and Tissue Homogenates after Injection of AT (Data from 20 Experiments)

Object	E, mmol H_2O_2/mg protein±30%	%
Mitochondria of intact animals	20.5	100
The same+AT	1.59	7.75
Homogenates of intact animals	7.1	100
The same+AT	0.07	10

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Effect of Sedatin, a Synthetic Dermorphin Analog, on Cell Division in the Corneal and Lingual Epithelia of Rats

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Sedatin, an analog of the opioid peptide dermorphin, which is a mixed μ- and δ-receptor agonist, had no effect on DNA synthesis and mitotic activity in the corneal or lingual epithelium of rats in a single dose 10 μg/kg, but stimulated cell division in both epithelia in a single dose of 100 μg/kg and in the 10 μg/kg dose administered either once daily over a 21-day period or just once after a single injection of the opiate receptor antagonist naloxone.

Key Words: *dermorphin; DNA synthesis; cell division*

As shown in our earlier study [3], the synthetic analogs of dermorphin that are superselective μ-receptor agonists inhibit cell division in corneal and lingual epithelia. One of those analogs, provisionally called sedatin, also displayed a high affinity for δ-receptors as compared to the previously tested dermorphin analogs [4]. The biological properties of sedatin, which is thus a mixed μ- and δ-receptor agonist, are currently being explored. We have also shown [2] that ligands for different receptor subpopulations exert differential effects on cell proliferation. Thus, the μ-receptor agonists DAGO and dermorphin inhibit DNA synthesis in corneal epithelium, whereas dalargin and DADL, which preferentially bind to δ-receptors, stimulate cell division in a number of epithelia.

The present study was undertaken to test the dermorphin analog sedatin for its effects on cell division processes in corneal and lingual epithelia (for it was not possible to decide *a priori* how it would influence cell proliferation). We hoped that the results of such a study would be of help in the projected development of pharmaceutical preparations based on this bioactive peptide.

MATERIALS AND METHODS

The study was conducted on 115 male rats weighing 160-190 g. The dermorphin analog sedatin used was

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