Changes in IDR activity in the liver and heart during starvation and muscular work and in the recovery period after muscular work are thus one mechanism of regulation of gluconeogenesis, glycogenolysis, and glycogen resynthesis.

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COMPONENTS OF THE GABA-ERGIC SYSTEM AND LIPID PEROXIDATION IN ACUTE EXOGENOUS ACRLONITRILE POISONING

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The organism responds to stress by a "sterotyped" form of biochemical, functional, and structural changes, the degree of which is assessed on the basis of changes in concentrations of hormones of the pituitary-adrenal system.

The attention of research workers is currently drawn to the study of cellular and molecular mechanisms of stress injuries in effector target organs. The role of the GABA system in limitation of the stress syndrome has been described and data published on the state of receptor membranes [10]. Hormone reception and barrier, detoxication (the cytochrome P-450 system), and other functions of cell membranes are easily disturbed during the action of products of lipid peroxidation (LPO), one of the most common mechanisms of injury to biomembranes that determines the effects of a number of physical and chemical factors [5]. The attention of research workers is thus beginning to be diverted toward other, as yet inadequately studied mechanisms of stress, but which are also, like changes in the endocrine system, general and nonspecific in character.

This paper describes the study of the state of some parameters of the system of the inhibitory neurotransmitter GABA, certain functions of cell membranes, and the possible role of LPO, and it also assesses the effectiveness of antioxidants in the prevention of stress injuries, in a model of poisoning by the widely used industrial monomer acrylonitrile (AN), during the production and use of which cases of acute poisoning have been observed [13].

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TABLE 1. Effect of AN (0.19 mmole/kg) on Concentrations of NA and Adrenalin (A) in Tissues (in nmoles/g) and Blood Plasma (in nmoles/ml) of Rats (M ± m)

Time after injection, h	Brain		Adrenals		Spleen		Plasma	
	NA	A	NA	A	NA	A	NA	A
Control	0,73±0,06	0,322±0,055	1620±54	1332±0,53	10,29±0,53	5,40±2,01	20,10±2,01	16,37±0,44
1	0,72±0,06	0,229±0,055	2323±49	1801±43	9,28±0,24	6,71±0,38	7,74±1,77	18,99±1,04
2	0,48±0,06	0,109±0,055	1572±65	1670±43	6,21±0,18	4,15±0,44	31,62±1,89	37,39±1,46
4	0,51±0,06	0,186±0,055	4735±102	2909±114	6,21±0,18	2,51±0,33	54,14±3,72	43,66±4,42
8	0,52±0,06	0,175±0,055	2909±109	1828±102	7,15±0,53	1,96±0,27	37,12±2,31	29,25±1,15
48	0,78±0,06	0,267±0,055	2873±127	2145±63	12,77±0,65	6,71±0,44	48,94±0,83	33,62±0,11
72	1,09±0,06	0,535±0,055	4546±83	4268±83	1,09±0,47	5,08±0,27	30,50±2,07	24,51±2,13

TABLE 2. Effect of Vitamin E on Changes in Some Biochemical Parameters in Acute AN Poisoning (M ± m)

Parameter	Time after injection of AN, h	Control	AN	Vitamin E + AN
GABA, μmoles/g tissue	1	0,18±0,01	0,15±0,01*	0,22±0,02
Glutamate decarboxylase, μmoles GABA/h/g	1	12,9±1,2	9,1±0,6*	13,1±1,9
Fructose monophosphate aldolase, μmoles/min/mg protein	2	0	0,06±0,01*	0
Cytochrome P-450, nmoles/mg protein	$2^1/_2$	0,7±0,1	0,4±0,1*	—
Diene conjugates, optical units/ml erythrocyte suspension	2	0,7±0,1	1,6±0,1*	0,9±0,1

*P < 0.05.

EXPERIMENTAL METHOD

Noninbred albino rats of both sexes weighing 150-250 g were used, with 6-33 animals in each series. Some animals received injections of aqueous solutions of AN (0.19 or 0.95 mmole mmole/kg, intraperitoneally) noradrenalin (NA; 10 μmoles/kg, subcutaneously), an oily solution of α-tocopherol (0.21 mmole/kg, intramuscularly, 3 times — 48, 24, and 2 h before the injection of AN), or the same volume of the corresponding solvent. Fructose monophosphate aldolase activity was determined in blood serum obtained from the caudal vein before the experiment began (background) and after injection of AN [3]. The intensity of LPO was judged from the level of diene conjugates in erythrocytes from the rats' blood [11]. Liver microsomes were obtained with the use of Ca^{++} [16]. The content of cytochrome P-450 in the microsome preparations was measured with the SF-10 spectrophotometer [9]. The GABA content in brain homogenates was determined chromatographically [8] and the concentration of amines in the tissues and plasma was determined fluorometrically [12]. Activity of brain glutamate decarboxylase [8] and phosphorylase [1] and of liver disulfide reductase [6] was studied. The effect of NA, NaF, and 3', 5' -AMP on disulfide reductase activity was studied as described previously [6]. The regulators were incubated in the presence of 1 mM theophylline and ascorbate with the postmitochondrial supernatant from liver obtained by centrifugation at 18000g, and which, if homogenized under "rigorous" conditions (1 min, 3000 rpm), contained not only disulfide reductase, but also fragments of plasma membranes not sedimented at the level of acceleration used [15]. Protein was determined by Lowry's method. The results were subjected to statistical anaysis by Student's t test.

EXPERIMENTAL RESULTS

Acute poisoning and AN caused a decrease in the concentrations of NA and adrenaline in the organs and the development of hyperadrenalinemia and hypernoradrenalinemia, which can be explained by the outflow of NA into the blood stream from sympathetic nerve endings of the tissues and simultaneous stimulation of catecholamine biosynthesis in the adrenals, where the amine concentration was consistently higher than the control values (Table 1). Probably stimulation of the central, mediator and hormonal divisions of the sympathico-adrenal system is concerned in the formation of adaptive reactions during AN poisoning.

As Table 2 shows, injection of AN led after 1 h to a fall in the GABA level in the brain, possibly due to inhibition of the enzyme of GABA biosynthesis, i.e., glutamate decarboxylase.

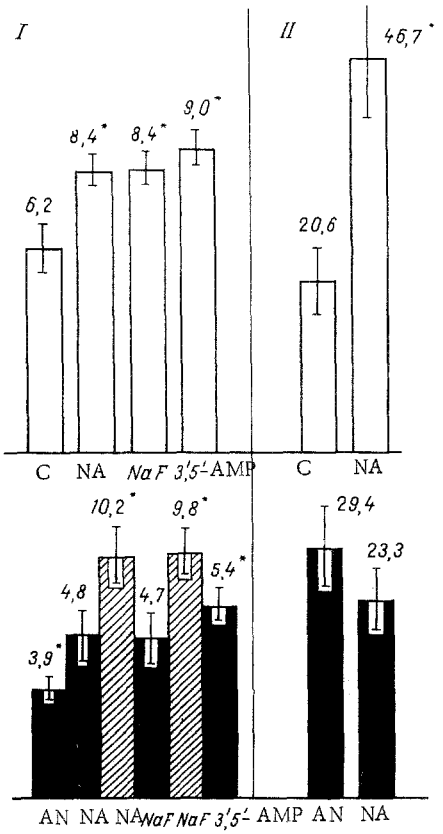

Fig. 1. Effect of acute AN poisoning on basal activities of disulfide reductase and phosphorylase of rat liver and realization of stimulating effects of various regulators. I) Disulfide reducatase activity (in nmoles/SH/mn/mg protein), II) phosphorylase activity (in μmoles P_{in}/min/g tissue). Unshaded columns represent intact rats, black columns rats poisoned with AN, obliquely shaded columns rats receiving AN after preliminary injection of α-tocopherol. C) Basal activity of enzyme, AN) acrylonitrile, NA) noradrenalin. *) Differences compared with control significant, **) difference compared with activity after AN significant.

The possible limiting role of GABA in the development of the stress syndrome [10], and also the fact that deviation in brain activity in some cases are the result of disturbances of relations between the levels of biogenic amines in different parts of the brain [2], suggest that some of the symptoms of AN poisoning (increased excitability, some degreen of hyperreflexia, turning into convulsions, ending in paralysis and death [13]) may be connected with the resultant effect of high concentrations of amines against the background of a lowered GABA level.

Evidence of injury to cell membranes in AN poisoning is given by the appearance of aldolase activity in the blood, for this is observed only when the permeability of the hypatocyte plasma membrane is disturbed [3], and also by a fall in the cytochrome P-450 content of the microsomes (Table 2). The membrane-toxic action of AN was confirmed by experiments with phosphorylase and disulfide reductase, that is, regulating enzymes of carbohydrate and thiol-di sulfide metabolism. It was found that although basal disulfide reductase activity was reduced in the liver of the poisoned rats (receiving 0.19 mmole AN/kg body weight), sensitivity of the enzyme was still preserved to the activating effect of 3',5'-AMP (Fig. 1), which acts distally to the plasma membrane at the protein kinase level [4]. However, effects of NA, which interacts with the adrenorecptor, and of NaF, which stimulates adenylate cyclase (both

proteins distributed in the lipid phase of the plasma membrane [15], were found to be lost. Definite stimulating effects were observed in the liver of intact rats at all levels of regulation.

In other experiments NA, which led to marked activation of phosphorylase after 20 min (Fig. 1), proved ineffective when given after AN (0.95 mmole AN/kg given 2 h before NA). The probability that the blocking action of AN was connected with previous phosphorylase activation on account of excitation of the sympathico-adrenal system could be discounted because in the combined AN + NA series neither summation nor preservation of the effect at the level of amine could be observed, as might have been expected had the effect of AN been mediated through catecholamines. At the same time, a samll increase, admittedly not significant, was observed in activity of the enzyme against the background of AN, yet another argument in support of disturbance of membrane function through the action of AN was given by the experiments with emotional stress, which exclude any possibility of toxic injury to the regulatory apparatus of the cell. Covering the rat's head by an opaque polyethylene mask led after 30 min tomarked activation of the pituitary-adrenal system: The blood 17-hydroxycorticosteroid level was raised, the ascorbate concentration in the adrenals lowered, and the tissue reserves of catecholamines mobilized, with their discharge into the blood stream [12]; evidence of preservation of the metabolic effects of these catecholamines was given by activation of disulfide reductase in the liver from 6.5 ± 0.7 nmoles SH/min/mg protein in the control to 11.9 ± 1.2 nmoles SH/min/mg protein in the experiment ($P < 0.01$) and in the spleen from 4.7 ± 0.9 to 10.8 ± 1.6 nmoles SH/min/mg protein ($P < 0.02$), respectively. The data indicate that the toxic effect of AN is located at the level of the membrane component of the regulating system and they point to a probable role of failure of this mechanism in the disturbance of realization of hormonal influences despite a normal or even raised hormone concentration in stress. The reduction of the pressor effect of catecholamines against a background of AN, observed previously [14], can be explained from this standpoint.

The hypothesis [7] of the possible role of LPO in the pathogenesis of AN poisoning was confirmed in experiments in which the content of initial peroxidation products (diene conjugates) was found to be increase 1 h after injection of AN (Table 2). The fact the the prooxidant effect of AN is connected with the pathological changes described above was well confirmed by blockade of the accumulation of diene conjugates by vitamin E, on the one hand, and by disturbances in the GABA system, high blood enzyme levels due to stress, and restoration of sensitivity of disulfide reductase to NA and NaF, on the other hand (Fig. 1; Table 2).

In conculusion it must be emphasized that the specific prestress, harmful action of chemical compounds proceeds in the earliest stages and is due to irreversible binding of their highly active metabolites, formed in the cytochrome P-450 system, as the writers showed previously for AN [7]. This stage follows a rapid course because of the extreme instability of products of this kind. Nonspecific phenomena develop later; a fall in the thiol level, stimulation of LP and damage to biological membranes, stress effects of the endocrine system. Realization of the mobilizing action of the latter system during poisoning largely depends on the state of the membrane components of the effector organs. If this situation is taken into account, detailed characteristics can be given of the time course of transition of the stress syndrome, as a stage of adaptation, into the stage of pathogenesis, and a basis is established for the development of ways of preventing stress injuries.

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EFFECT OF THYROIDECTOMY AND HYPERTHYROIDISM ON ACTIVITY OF $Ca^{++}/2H^{+}$ ANTIPORTER ACTIVITY IN RAT LIVER MITOCHONDIRA

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Ca^{++} ions are a universal regulator of metabolic processes [9, 10]. One of the mechanisms regulating the concentration of ionized Ca^{++} in the cytosol is its transport Ca^{++} ions *in vivo* and *in vitro* [1, 2, 11]. It can be tentatively suggested that hormones can exert their action on metabolism by changing the distribution of Ca^{++} ions between mitochondria and cytosol. It has recently been shown that Ca^{++} transport in the the liver mitochondria is effected by two carriers: The Ca^{++} ion porter is responsible for electrophoretic transport from cytosol into mitochondria, and the $Ca^{++}/2H^{+}$ antiporter is responsible for removal of Ca^{++} ions from mitochondria in exchange for H^{+} ions [6, 7].

Since changes in activity of mitochondrial $Ca^{++}/2H^{+}$ antiporter during changes in the hormonal status of the body may affect the Ca^{++} concentration in the cytosol, the effect of thyroidectomy and hyperthyroidism on activity of $Ca^{++}/2H^{+}$ antiporter was investigated in rat liver mitochondria.

EXPERIMENTAL METHOD

Mitochondria from rat liver were isolated in 0.3 M sucrose containing 5 mM Tris-HCl, pH 7.4, at 5000g. Transport of Ca^{++} ions in the mitochondria was measured by an ion-selective Ca^{++}-sensitive electrode and pH-metric method based on the kinetics of Ca^{++}/H^{+} exchange in the presence of phosphate. The kinetics of swelling of the mitochondria was measured as changes in their optical density of 540 nm. Male rats weighing 100 g were used in experiments with thyroidectomy [3]. The thyroidectomized rats 4 months after the operation weighed 130-160 g, the controls 250-280 g. Hyperthyroidism was induced by intraperitoneal injection of thyroxine in a dose of 100 μg/100 g daily for 4 days.

EXPERIMENTAL RESULTS

After thyroidectomy the calcium capacity of rat liver mitochondria (the number of Ca^{++} ions accumulated by mitochondria before the beginning of spontaneous outflow of Ca^{++} from the mitochondria) was increased. Injection of physiological concentrations of thyroxine into thyroidectomized rats reduced the calcium capacity pratically to normal, but in hyperthyroidism the calcium capacity was significantly lower than in the control (Fig. 1). As Fig. 1b shows, adequate correlation exists between changes in the calcium capacity of the liver mitochondria and the rate of outflow of Ca^{++} ions from the mitochondria on additon of ruthenium red. Since the outflow of calcium ions is undertaken by $Ca^{++}/2H^{+}$ antiporter, which is insensitive to ruthenium red [6, 7], it can be concluded that after thyroidectomy manifestation of

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