EFFECT OF SUCCINATE DERIVATIVES ON CATECHOLAMINE LEVELS IN RAT ORGANS DURING COOLING

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The role of catecholamines (CA) in regulation of the stress response to cold is known. Excitation of the sympathicoadrenal system and increased secretion of CA caused by contraction of superficial vessels and reduction of heat loss are among the first mechanisms of defense in acute chilling. CA also activate mobilization of the energy resources and their utilization through intensification of oxidative reactions. The resistance of the body to the cold factor is correspondingly increased. The importance of adrenergic reactions is confirmed by the fact that drugs causing positive modulation of function of the adrenergic synapse significantly increase the resistance of animals to acute chilling [1, 2]. These data indicate that investigation of the state of the sympathicoadrenal system during pharmacologic correlation of resistance under conditions of chilling is an essential component in the evaluation of the effectiveness of the drugs used for these purposes.

The aim of this investigation was to study the time course of the CA levels in the organs of rats during acute exposure to low temperatures, and treated with preparations of the actoprotector group: tonibral (a polyester of dimethylethanol and succinic acid) and another succinic acid derivative – IOKh-13. It was shown previously that preliminary administration of succinic acid considerably increased the resistance of animals to acute cooling due to activation of reactions of energy metabolism [1].

EXPERIMENTAL METHODS

Experiments were carried out on 40 noninbred male rats weighing 140-150 g, divided into four groups: 1) intact animals, 2) controls (chilling for 48 h), 3) rats receiving IOKh-13 1 h before cooling, 4) rats receiving tonibral 1 h before cooling. Cooling was carried out by Le Blanc's method [5] in a climatic chamber (−15°C), the animals being kept separately in constraining cages, allowing relative preservation of their mobility. Rats of the experimental groups received IOKh-13 and tonibral intraperitoneally in dose of 80 and 64 mg/kg respectively. After cooling for 48 h the rats were decapitated and their organs (heart, liver, adrenals, skeletal muscle) were quickly removed and fixed in liquid nitrogen. A weighed sample of frozen tissue was homogenized with 0.4 N $HClO_4$ in the ratio of 1:9. The supernatant was obtained, treated by the method in [6], and CA were determined by means of an MPF-4 spectrofluorimeter (Hitachi, Japan) and standard solutions of adrenalin, noradrenalin – NA (acid tartrates), and dopamine – DA (hydrochloride, from Serva, West Germany).

The numerical data were subjected to statistical analysis. Differences were considered significant at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

The CA level in the organs of noninbred rats during cooling (−15°C) with relative preservation of mobility, showed changes in both control and experimental animals (Fig. 1).

Cooling of the control rats for 48 h led to a marked and persistent decrease in the CA concentration in most tissues, evidence of insufficiency of the sympathicoadrenal system,

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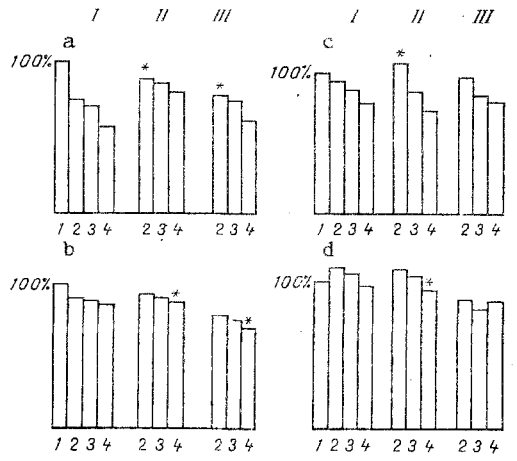

Fig. 1. Adrenalin, NA, and DA levels in rat tissues during cooling (in % of levels in intact animals). a) Heart, b) liver, c) muscle, d) adrenals. 1) CA concentration in intact animals; 2) adrenalin concentration; 3) NA concentration; 4) DA concentration. I) Control; II) IOKh013; III) tonibral. *$P \leq 0.05$ compared with intact animals.

exhaustion of CA reserves, and the development of decompensation of temperature homeostasis. Altogether 74% of the total number of animals in the control group died from hypothermia at this time. A similar change in the CA level in the rats' organs after 48 h of cooling by comparison with that in intact animals also indicates disturbance of release and functioning of endogenous CA, which essentially abolish temperature regulating reactions and reduce resistance to cold.

However, the adrenalin and, in particular, the NA levels in a thermogenic tissue (muscle) of the control rats differed only a little from their levels in intact animals, evidence that during this period the resistance of the surviving animals to cooling was maintained through activation of muscle thermogenesis. An increase in the adrenalin and NA concentrations, accompanied by a fall in the DA level, were observed in a hormone-producing organ (the adrenals) during exposure for 48 h to cold.

Cooling rats to −15°C, with relative preservation of their mobility, thus induced a distinct response of the sympathicoadrenal system: a tendency was observed for the CA concentration in the heart to fall. In muscles, however, intensive exhaustion did not take place (adrenalin, NA), indicating that the protective effect is exhibited through activation of muscular thermogenesis.

Injection of IOKh-13 and tonibral significantly changed the CA level in the rats' organs and was accompanied by a sharp increase in the NA and adrenalin concentrations in the muscles, evidence of maintenance of resistance to cooling both through activation of muscular thermogenesis and through the adrenergic component, behaving as a second line of defense against cold. In addition, elevation of the adrenalin level was observed in the liver of the experimental rats (Fig. 1). It can be tentatively suggested that in this case (during treatment with the compounds) the CA reserves were exhausted more slowly than in the control, and for that reason the survival rate was higher in animals treated with IOKh-13 (56%) and tonibral (40%). The fact that IOKh-13 was more effective than tonibral corelates with the higher adrenalin and NA levels in the heart muscle, which evidently maintained a higher level of contractility and reserve capacity of the heart [4].

Data on the CA concentration in the rats' organs during administration of substances concerned with energy metabolism under conditons of relative preservation of their mobility are evidence of activation and enhancement of resistance to cold and they indicate that, in principle, drugs belonging to the actoprotector class can be used for pharmacologic modification of resistance to acute chilling.

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ROLE OF PHOSPHORIC ACID RESIDUES IN THE FORMATION OF ANTIGENIC DETERMINANTS OF DNA STRUCTURAL COMPONENTS

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Antibodies to DNA were first found in patients with systemic lupus erythematosus (SLE) and for a long time their appearance was regarded as one of the most important signs of this disease. Subsequently antibodies interacting with various structural components of the nucleus, and carrying under physiological conditons a strong negative (DNA, RNA) or positive (histones) charge, were discovered in patients with SLE. By the use of monocloncal antibodies to DNA, synthesized by hybridomas created on the basis of spleen cells of mice with an autoimmune syndrome, it was found that they react with various macromolecules carrying a negative charge (for example, with proteoglycans) which are structures of cell membranes [3, 4].

These data suggest that the antigenic stimulus for the formation of naturally found antibodies, reacting with DNA, in some cases may be structures carrying a positive or negative charge under physiological conditons.

In the investigation described below the role of phosphoric acid residues was studied in interaction between antibodies induced to structures carrying a positive charge (thymidine) and a negative charge (denatured and UV-modified DNA).

EXPERIMENTAL METHODS

Antithymidine sera were obtained by immunization of rabbits with conjugates of ribosylthymine with bovine serum albumin, prepared by the method in [2]. Antisera to denatured DNA and UV-irradiated DNA were obtained by the method in [6]. As other sources of antibodies, sera from patients with SLE, under treatment in the Institute of Rheumatology, Academy of Medical Sciences of the USSR (Moscow), were used.

Antigens for radioimmunoassay were tritium-labeled thymidine and DNA isolated from *E. coli* W3110 thy^- cells by a modified method [5], with specific activity of 80,000 cpm/μg.

As inhibitors of the antigen-antibody reaction we used thymidine, thymidine triphosphate (TTP, from Calbiochem, USA), thymidyl tri-, hexa-, and octanucleotides – T_3, T_6, and T_8 respectively ("Biochemicals," USA), and also thymine and thymidine dimers, UV-irradiated DNA, and UV-irradiated DNA subjected to hydrolysis down to bases.

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