The Effect of Delta-Sleep-Inducing Peptide on the Ca-Transporting System of the Sarcoplasmic Reticulum and Activity of the Myocardial Antioxidant Enzymes

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It is demonstrated that immobilization stress against the background of lowered catalase activity impairs the function of the sarcoplasmic reticulum Ca pump, particularly at high Ca^{2+} levels. The membranes of intracellular Ca^{2+} depots are destroyed much more rapidly than in the control, which results in Ca^{2+} release. Administration of delta sleep-inducing peptide to control animals results in a 30% increase in catalase activity for an unchanged level of superoxide dismutase and markedly improves the function of the Ca-transporting system at elevated levels of free Ca^{2+}. A long-term stress after administration of the peptide not only causes no damage to the Ca-transporting system but actually increases its efficiency (compared with the control) at a high catalase level.

Key Words: *delta sleep-inducing peptide; Ca pump; sarcoplasmic reticulum; superoxide dismutase; myocardium*

Stress-induced damage to myocardial ion-transporting systems: sarcolemmal Na,K-ATPase [6] and sarcoplasmic reticulum Ca-ATPase [13] resulting from the activation of lipid peroxidation (LPO) can be prevented by central and peripheral stress-limiting systems of the organism [5]. Adaptation to a short-term stress, which can prevent disturbances of the electrical stability of the heart and arrhythmias in stress, ischemia, reperfusion, myocardial infarction, and post-infarction cardiosclerosis [14], is an example of combined activation of the central and peripheral stress-limiting systems of the organism. Adaptation to stress also prevents stress-induced damage to the Na,K pump [6] and optimizes the function of the Ca pump [13], i.e., it triggers a mechanism that operates at the level of the heart, limits arrhythmias, and protects against alterations in Ca homeostasis.

An important role of delta sleep-inducing peptide (DSIP) in adaptation has recently been recognized. It has been shown that, in addition to normalizing sleep, this peptide activates the monoaminergic transmitter and serotoninergic systems and, being an opiate receptor agonist, displays antidepressant and analgetic activities [3,11]. DSIP raises the resistance of animals to emotional stress [7], prevents their death from cardiovascular disorders [4], and elicits an antiarrhythmic effect in emotional stress [8]. Moreover, it prevents stress-induced sleep disorders in rabbits [15]. Administration of DSIP and its analogs forestalls activation of LPO in acute pancreatitis and cold stress and stabilizes lysosomal membranes [10]. Thus, DSIP elicits both central and peripheral effects, regardless of the route of administration. However, its effect on the electrical stability of the heart devel-

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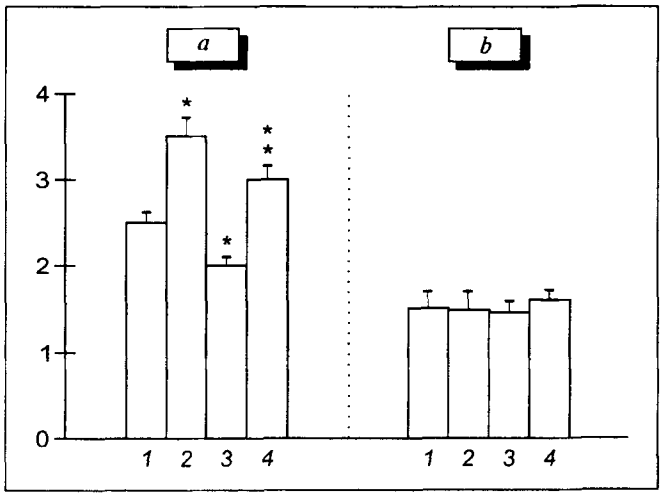

Fig. 1. Activity of antioxidant enzymes: catalase (*a*) and superoxide dismutase (*b*). *1*) control; *2*) delta sleep-inducing peptide; *3*) stress; *4*) DSIP+stress. Ordinate: *a*) catalase activity, nmol H_2O_2/min×mg protein; *b*) superoxide dismutase activity, U/mg protein. *$p<0.01$, **$p<0.05$ compared with the control.

ops much later after central than after intravenous administration [1].

The peripheral effect of DSIP on Ca homeostasis of the myocardium, which suffers considerably during stress, has not been studied. Our goal was therefore to evaluate the protective activity of DSIP against stress-induced damage to the myocardial Ca-transporting system and to compare it with the activity of myocardial antioxidant enzymes.

MATERIALS AND METHODS

Male Wistar rats weighing 200 g were used. They were assigned to 4 groups, 8 animals in each: group 1 served as a control, group 2 rats were subjected to immobilization stress (fixation by all four limbs on the back for 1 h), group 3 rats received intraperitoneal injections of 60 nmol/kg DSIP, and group 4 rats were exposed to immobilization stress 30 min after the administration of DSIP. Immediately after the stressing session, the animals were euthanized (40 mg/kg Nembutal), and the hearts were collected, washed in ice-cold normal saline, and frozen in liquid nitrogen.

The activities of catalase and superoxide dismutase were measured by methods described in the literature [12] and [9], respectively. The rate of Ca^{2+} transport in homogenates was determined in an Orion EA 940 ionometer with a Ca^{2+}-selective electrode [13] from the rate of adsorption of added Ca^{2+} by vesicles of the sarcoplasmic reticulum (SPR) during 5 min in a temperature-controlled chamber with stirring. The homogenate (50-200 μl) was added to 5 ml medium containing 100 mM KCl, 15 mM K oxalate, 20 mM HEPES (pH 7.0), 4 mM $MgCl_2$, and 5 mM NaN_3. ATP and $CaCl_2$ were added beforehand to final concentrations of 4 mM and 7-20 μM, respectively.

Oxidation *in vitro* was induced by $FeSO_4$ (10 μM) plus ascorbate (0.2 mM) at 37°C and a protein concentration of 2 mg/ml in the incubation medium containing 20 mM Tris-HCl, and 10 mM KCl (pH 7.4 at 37°C). The protein concentration was measured using the fourth derivative of the absorbance spectrum (320-240 nm) in a medium containing 20 mM histidine (pH 7.2), 50 mM NaCl, 8.1% sodium dodecyl sulfate, and the homogenate. The results were analyzed using Student's *t* test.

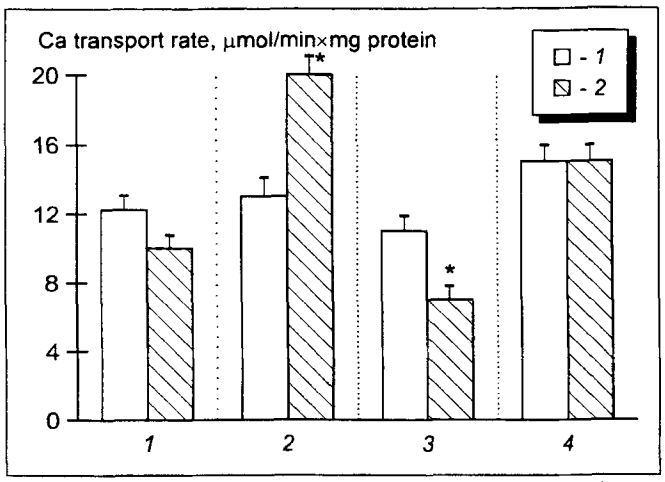

Fig. 2. Effect of an increase in the concentration of unbound calcium on the function of the Ca-transporting system of the sarcoplasmic reticulum. *I*) rate of Ca transport at 7 μM free Ca in the medium; *II*) rate of Ca transport at 20 μM free Ca in the medium. *$p<0.02$ compared with *I* in groups 2 and 3. Here and in Fig. 3: *1*) control; 2) administration of DSIP; 3) after immobilization stress; *4*) after stress plus DSIP.

RESULTS

First, we evaluated the activity of antioxidant enzymes after stress and administration of DSIP. The activity of superoxide dismutase did not change during stress (Fig. 1), while the activity of catalase decreased 22%. In this case, a decrease in enzyme activity does not indicate an adaptive modification in response to a decrease in the intensity of LPO, as, occurs, for example, at a lowered partial pressure [14], but, on the contrary, points to excessive activation of LPO [5] and a considerable accumulation of hydrogen peroxide. Since LPO is confined to the membrane and the efficiency of Ca transport correlates with the intensity of LPO [2], such a decrease in the activity of catalase can be expected to impair the function of the SPR Ca-transporting system.

As shown in Fig. 2, the transport rate in the SPR drops after stress. Moreover, an increase in the concentration of exogenously added Ca^{2+} from 7 to 20 μM results in an additional 30% inhibition of the activity of Ca transport after stress, indicating a reduced

ability of the Ca-transporting system in the SPR to remove Ca from the myoplasm when this is crucial (at high Ca concentrations). Thus, a decrease in the rate of Ca absorption by the SPR vesicles leads to an increase in the free intracellular Ca concentration, which inhibits the Ca pump, i.e., a vicious circle is formed [5]. In addition, if the initial concentration of free Ca^{2+} after stress is the same as in the control, a long-term incubation upon LPO induction *in vitro* activates LPO in the SPR and mitochondria, resulting in membrane damage and Ca^{2+} release. In the control, the concentration of unbound Ca^{2+} does not change significantly after a long-term incubation with the oxidation system but increases more than 35% after stress (Fig. 3). Taken together, these findings indicate that the functioning of the Ca pump is impaired and the membranes of the intracellular Ca^{2+} depots are damaged as a result of stress.

The activity of catalase increased 30% and that of superoxide dismutase remained unchanged after administration of DSIP to control animals (Fig. 1). The rate of Ca^{2+} transport in the SPR did not differ significantly from the control (Fig. 2). It is noteworthy that long-term incubation in the oxidation system induced no additional Ca^{2+} release from the intracellular depots as occurred in stress (Fig. 3), indicating that the membranes are protected against LPO induced *in vitro* even by strong oxidants. When the concentration of exogenously added Ca^{2+} was raised from 7 to 20 μM (Fig. 2), the rate of Ca^{2+} transport was not only not inhibited by the high Ca^{2+} concentration but actually rose more than 40%. This indicates that the functioning of the SPR Ca-transporting system is markedly improved at high concentrations of calcium. Interestingly, similar results were obtained only in one model: the use of adaptation to repeated stress to prevent stress-induced damage to the membrane-bound Ca-transporting system of the SPR [13]. Just as with the effect of DSIP, adaptation to stress improves the efficiency of the SPR Ca pump at high Ca^{2+} concentrations, indicating a possibility of rapid compensation for the elevated levels of unbound Ca^{2+} in the myocardium. The protective effect of DSIP is very strong, since further stress after administration of this peptide does not impair Ca transport but even improves some of its parameters: the rate of transport is 30% higher than in the control or after administration of DSIP and is not inhibited by high Ca^{2+} concentrations, and Ca^{2+} release due to long-term oxidation is not higher than in the control and considerably lower than in stress (Figs. 2 and 3). It is noteworthy that all these modulations went along with increased catalase and superoxide dismutase activity (Fig. 1). An increase in the activity of superoxide dismutase occurred only in the

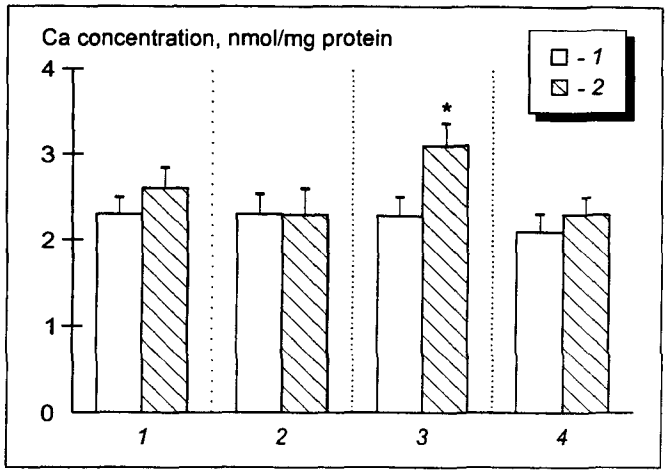

Fig. 3. Release of unbound Ca from intracellular Ca depot as a result of incubation at 37°C. *I*) zero time point; *II*) after 1 h 50 min of incubation. **p*<0.02 compared with *I* in group *3*.

"stress+DSIP" group, while in control animals it remained unchanged after DSIP administration. In this connection it is important to note that DSIP differs from other hypnogenic preparations in that it does not have a sedative effect and does not manifest its properties in situations and at times unusual for sleep, does not alter the structure of sleep in people without sleep disorders, and manifests higher activity in severe sleep disorders [11].

In this case the peripheral effect of DSIP shows a similar feature: the peptide by itself only slightly changes the antioxidant protection and Ca transport in the SPR, whereas after stress it elicits a pronounced protective effect.

Thus, DSIP prevents stress-induced disorders in myocardial Ca homeostasis, stabilizing the contractile function of the heart in acute stress. This peptide limits the stress reaction at the central level and has a peripheral effect on the Ca transport system in the myocardial SPR. It should be stressed that this effect is similar to the stabilizing effect on Ca transport achieved by adaptation to stress factors which limit the activation of the stress-realizing systems.

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Effects of Adaptation to Periodic Hypoxia on Kinetic Parameters of Respiratory Chain Enzymes in Rat Brain

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A study of kinetic parameters of brain respiratory enzymes revealed that the maximal velocity and the Michaelis apparent constant for NADH-cytochrome C-reductase are significantly lower in low-resistant rats than in rats with a high resistance to hypoxia. Adaptation to periodic hypoxia increases total resistance only in low-resistant rats. It is accompanied by an increase in the values of kinetic parameters for NADH-cytochrome C-reductase and cytochrome oxidase. Kinetic parameters for these enzymes in the brain of high-resistant rats are either unaltered or even decreased. It is suggested that the first enzymatic complex of the respiratory chain is one of the limiting or regulating links in energy metabolism determining the brain's resistance to hypoxia.

Key Words: *adaptation; hypoxia; enzymatic activity; respiratory chain; individual resistance*

We have shown that long-term periodic adaptation (LTA) to hypoxia is accompanied by a transformation in the activity of the brain respiratory chain which manifests itself in suppressed respiration and intensified oxidative phosphorylation, altered oxidative activity of respiratory substrates [3], a decreased concentration of cytochromes, and an increased number of brain mitochondria [2]. These data suggest that LTA modifies the activity of the respiratory chain enzymes. As no systematic data are available on this subject, in the present study we investigated the effects of LTA on the kinetic parameters of some enzymes of the brain respiratory chain in rats with various degrees of resistance to oxygen deficiency.

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MATERIALS AND METHODS

The study was carried out on outbred male rats weighing 180-200 g. The animals were divided into high- and low-resistant groups (HR and LR, respectively) according to their resistance to acute hypoxia. Periodic LTA to hypoxia was produced by a technique described earlier [6]. Rats were decapitated and homogenates

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