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ROLE OF BLOOD LIPOPROTEINS IN ADAPTIVE CHANGES IN RAT LIVER MITOCHONDRIA

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When the body is under intensive functional loading the liver mitochondria undergo complex structural and functional changes, an integral parameter of which is their reversible swelling. Its mechanism has not yet been explained. Adaptive hormones (glucocorticoids, catecholamines) have no direct effect on the mitochondria, and for that reason the action of their mediators is currently under active discussion. Among the latter an important role is played not only by cyclic nucleotides [1] but also, probably, by lipoproteins (LP). Under the influence of glucocorticoids the blood LP level rises [2].

Penetration of lipoprotein particles into hepatocytes takes place through the activity of highly specific receptors located on the outer surface of the plasma membranes [10]. Liver cells, which occupy key positions in metabolism of all classes of LP, can bind LP of all densities to their receptors and ingest them [15]. The study of the intracellular distribution of LP whose protein component was labeled with radioactive iodine, in experiments *in vivo* [7] and *in vitro* [6], showed the label to be present not only in the fraction of secondary lysosomes, where the final stages of degradation of lipoprotein particles take place, but also in fractions of nuclei, mitochondria, and microsomes. These facts provide a solid basis for explaining the effect of serum LP and their apoproteins on the activity of a wide range of biochemical processes taking place in the extracellular space and inside the cell [2, 4, 5, 8, 12-14].

Activation of serum LP metabolism under conditions of stress may have a significant effect on the state of oxidative phosphorylation in the liver mitochondria. It was shown previously [3] that different classes of blood LP, as well as their apoproteins, can activate mitochondrial ATPase in intact and starving rats and rats subjected to intensive physical exertion.

The object of this investigation was to determine the possible role of blood LP and their apoproteins in initiation of swelling of liver mitochondria as one stage in their adaptive changes during functional stress. The comined effect of apoproteins and cAMP, which has a specific effect on individual functions of mitochondria [1], on the swelling process also was investigated.

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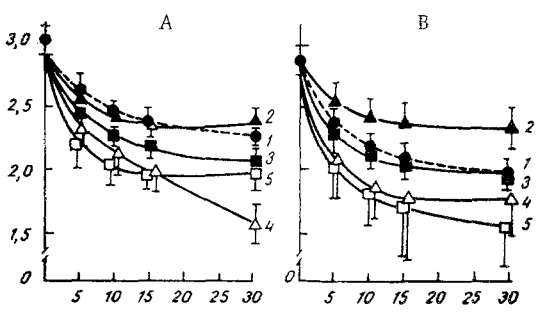

Fig. 1

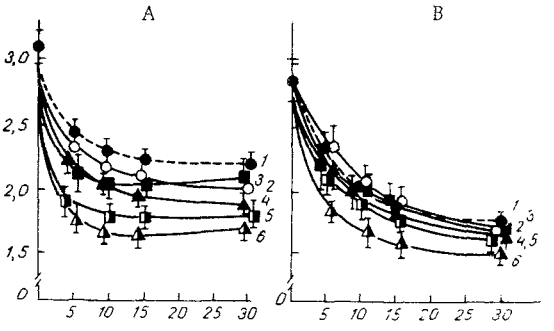

Fig. 2

Fig. 1. Effect of blood LP and their apoproteins on swelling of rat liver mitochondria (data of eight experiments). Abscissa, incubation time (in min); ordinate, optical density units at 520 nm/mg protein of rat liver mitochondria. A) Control, B) starvation. 1) No additives, 2) 135 μg VLDLP/mg mitochondrial protein, 3) 213 μg HDLP/mg mitochondrial protein, 4) 168 μg apo-VLDLP/mg mitochondrial protein, 5) 196 μg apo-HDLP/mg mitochondrial protein.

Fig. 2. Effect of apoproteins of different classes and of dibutyryl-cAMP on swelling of rat liver mitochondria. 1) No additives, 2) dibutyryl-cAMP, 3) apo-HDPL, 4) apo-VLDLP, 5) dibutyryl-cAMP + apo-HDLP, 6) dibutyryl-cAMP + apo-VLDLP. Doses of apoproteins of both classes in all cases were 46 μg/mg mitochondrial protein; final concentration of dibutyryl-cAMP was 10^{-6}. Remainder of legend as to Fig. 1.

EXPERIMENTAL METHOD

Male Wistar rats weighing 130-170 g were used. Starvation for 72 h, with water for drinking, was used as the stressor. LP were isolated from the blood serum of intact rats by differential centrifugation [11] on the Beckman model L5-75 ultracentrifuge with 75 Ti rotor (USA). The LP fraction was dialyzed against 0.15 M NaCl solution for 48 h at 4°C. Apoproteins were obtained by delipidization of the corresponding LP with a mixture consisting of equal volumes of chloroform and methanol, which was added to the LP in the ratio of 20:1 by volume. The precipitate of apoproteins was dissolved in 20 mM Tris-HCl, pH 7.4. The procedure of isolation of mitochondria from rat liver and the method of determination of mitochondrial protein, LP, and apoproteins were described previously [3]. The effect of blood serum LP, their apoproteins, and of dibutyryl-cAMP on the swelling process was judged by charges in optical density of a mitochondrial suspension measured at 520 nm on a Hitachi model 200-20 spectrophotometer (Japan), with constant mixing in an incubation medium of the following composition (in mM): KCl 125, KH_2PO_4 5, $MgCl_2$ 5, Tris-HCl 20, pH 7.4. Dibutyryl-cAMP was added to the medium in a final concentration of 10^{-6} M [1]. The quantity of LP and apoproteins added is indicated in the captions to Figs. 1 and 2.

EXPERIMENTAL RESULTS

The optical density of the suspension of freshly isolated mitochondria from the liver of the hungry rats was lower than that of the suspensions obtained from intact animals. This is evidence of swelling of the organelles taking place *in situ* under the influence of starvation, and preserved in the mitochondria after isolation. However, the fall in optical density by comparison with the control was not statistically significant (a reduction by 4 and 6.8% respectively; Figs. 1 and 2). These facts indicate that swelling of mitochondria during starvation is a reversible process of low amplitude. This conclusion agrees with data obtained previously [2, 9]. LP and dibutyryl-cAMP, if added separately, had no effect on the kinetics of the swelling process in either the control or the experimental series. High-density apoproteins (apo-HDLP) and apoproteins of very low density (apo-VLDLP), unlike intact LP, stimulated swelling. These properties were most clearly marked in apo-VLDLP, under the influence of which the optical density of a suspension of mitochondria from intact rats was significantly reduced after incubation for 30 min (Fig. 1A, Fig. 2A). As a result of the combined action of dibutyryl-cAMP and apoproteins on liver mitochondria from control and hungry rats, the swelling increased more than with apoproteins alone. Maximal stimulation of swelling was observed through the combined effect of apo-VLDLP and cAMP. Swelling

of liver mitochondria from the control rats was significantly increased by the combined action of the effectors throughout the incubation period ($P < 0.01$), whereas swelling of the mitochondria of previously starved animals was observed after 5 and 30 min of incubation (Fig. 2A, B). Significant stimulation of swelling through the action of a combination of apo-HDLP and cAMP was observed only in experiments with mitochondria from intact rats after 5, 15, and 30 min (Fig. 2A).

The data described above show that, first, a component of the blood LP, especially apo-VLDLP, had a considerable influence on the swelling of the mitochondria, for delipidization intensified the swelling process, and second, stimulation of swelling under the influence of apoproteins or a combination of apoproteins with cAMP was manifested most clearly on liver mitochondria obtained from control rats. Exidently on contact with "intact" mitochondria apoproteins are more able to realize this effect than during interaction with mitochondria obtained from starved animals.

The protein component of serum LP which actively penetrates into the hepatocyte cytoplasm during stress, may thus take part in adaptive changes in the mitochondrial apparatus of an animal under conditions of intensive functional strain.

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