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HIGH SENSITIVITY OF MITOCHONRDIA IN THE RAT SMALL INTESTINAL MUCOSA TO ADRENALIN

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KEY WORDS: mitochondria; small intestinal mucosa; oxidative phosphorylation; calcium capacity; adrenalin.

Most research into the effect of adrenalin on mitochondrial metabolism has been undertaken on mitochondria of the liver, which constitute a convenient and stable preparation [3, 5, 8, 15]. Functional characteristics of mitochondria of other, more reactive tissues and the effect of regulating factors on them have received much less study. One of the most reactive tissues is the small intestinal mucosa (SIM), which is sensitive to changes in the sympathicoadrenal system [6]. Probably on account of its increased reactivity, it is difficult to isolate mitochondria from this tissue [1].

In the investigation described below this difficulty was overcome by using new techniques to isolate mitochondria and to work with them. In this way the action of adrenalin on mitochondria of SIM and also on more native liver mitochondria could be studied.

EXPERIMENTAL METHOD

During work with the animals and isolation of mitochondria from the liver and SIM, a combination of conditions was adopted whereby organelles with properties closest to native could be obtained [1, 9]. Experiments were carried out on male Wistar rats weighing 200-220 g. The final dilution of the suspension during keeping was 70-80 mg protein/ml for liver mitochondria and 30-40 mg protein/ml for SIM mitochondria.

The effect of adrenalin on energy metabolism was assessed by pH-metric recording of oxidative phosphorylation [4] and of Ca^{++} uptake in mitochondria [13]. The incubation medium for the liver mitochondria (26°C) consisted of 4-5 mg mitochondrial protein in 1 ml, 150 mM sucrose, 50 mM KCl, 1 mM KH_2PO_4, and 3 mM Tris-HCl, pH 7.4; for SIM mitochondria (28°C) it consisted of 2-2.5 mg mitochondrial protein in 1 ml, 250 mM sucrose, 40 mM KCl, 3 mM KH_2PO_4, and 5 mM Tris-HCl, pH 7.4. Succinate (6 mM) was used as oxidation substrate. The pH of the incubation medium was recorded with an LPU-01 pH-meter and EZ-2 automatic writer. The action of adrenalin on the rate of phosphorylation of added ADP [14], the rate of uptake of Ca^{++}, and the calcium capacity (CC) of the mitochondria was studied [13]. Protein was determined by Lowry's method. Adrenalin was injected intraperitoneally 15 and 30 min and 3 h before sacrifice of the animals, in a dose of 5 μg or 25 μg per 100 g body weight. The β-adrenoblocker inderal, in a dose of 1 mg/100 g body weight, was injected intraperitoneally 30 min before injection of the hormone. Control animals received an injection of an equal volume of 0.9% NaCl solution at the corresponding times.

The results were subjected to statistical analysis by the comparison of pairs method [2].

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TABLE 1. Development of Stimulating Effect of Adrenalin on Oxidative Phosphorylation (in nanomoles ATP/mg protein/min) in Mitochondria from Rat SIM and Liver (M ± m, n = 5)

Time of action of adrenalin	SIM			Liver		
	control	experiment	changes, %	control	experiment	changes, %
			Adrenalin, 5 μg/100 g			
15 min	50,4±5,7	60,4±6,0	120*	87,6±5,6	107,7±9,4	123*
30 "	48,3±4,4	60,5±5,4	125*	88,0±5,9	110,1±10,3	127*
3 h	46,3±4,6	49,8±3,4	109	88,2±6,4	90,0±8,4	102
15 min (inderal)	52,5±6,2	55,4±4,3	106	89,8±7,3	88,9±7,8	99
			Adrenalin, 25 μg/100 g			
15 min	47,6±3,1	71,7±6,8	151**	86,2±9,4	112,0±7,2	130*
30 min	45,7±3,5	67,1±6,4	147**	86,9±7,0	100,4±11,7	115
3 h	47,5±4,5	57,0±2,9	120*	87,6±5,5	85,8±7,1	98
15 min (inderal)	47,2±5,2	53,3±5,4	113	89,2±5,7	88,9±5,6	100

Legend. *P < 0.05, **P < 0.01 compared with control.

EXPERIMENTAL RESULTS

The native character of isolated suspensions of mitochondria from the liver and SIM enabled their properties to be compared under different physiological conditions and, in particular, after injection of adrenalin, a powerful activator of energy metabolism, into the animal. Changes observed in the velocity of phosphorylation of added ADP in liver and SIM mitochondria during oxidation of succinate are given in Table 1, which shows values of the velocity of phosphorylation of the first quantity of ADP added, so that changes in the oxidative phosphorylation system could be detected by a greater degree than subsequent changes. As Table 1 shows, a physiological dose of adrenalin (5 μg/100 g) caused an approximately equal increase in the rate of phosphorylation as early as 15 min after injection compared with the control in liver and SIM mitochondria (23 and 20% respectively). After 30 min the activation effect remained at about the same level, and after 3 h normalization of the process was observed in both cases. Injection of adrenalin into rats 15 min before sacrifice, after preliminary β-adrenoreceptor blockade by inderal, abolished the activating effect of adrenalin in mitochondria from both tissues.

A fivefold increase in the dose of adrenalin potentiated the effect of an increase in the rate of phosphorylation 15 min after injection. However, whereas in liver mitochondria the velocity of phosphorylation increased only by 7%, in mitochondria of SIM it increased by 31% compared with the rate after injection of the smaller dose. After 30 min the stimulating effect of adrenalin was reduced in the liver, but in SIM it continued at the same level as that observed after 15 min of activation. The rate of phosphorylation in the SIM mitochondria of the experimental animals 3 h after injection of the larger dose of adrenalin was 20% greater than in the controls, whereas in the liver an activation effect could no longer be observed at this time. Inderal, as Table 1 shows, completely blocked the 15-min activation effect caused by injection of the large dose of adrenalin only in the liver, whereas in SIM mitochondria the adrenalin effect was only partially removed by inderal. Activation of oxidative phosphorylation in the liver is probably mediated principally through β-adrenoreceptors, whereas in SIM a more complex receptor structure is involved.

Mitochondria are important regulators of the intracellular calcium concentration. In SIM, "rapid penetration of Ca^{++} into the mitochondria and the consequent creation of a sink of these ions from the intestinal contents into the cell accelerates the process of Ca^{++} uptake in the intestine" [4]. Investigations [11, 12] using labeled ^{45}Ca during perfusion of organs and incubation of cells with adrenomimetics showed that calcium metabolism in the mitochondria of the heart and liver is controlled by the sympathicoadrenal system. We studied the action of adrenalin in vivo on the maximal quantity of Ca^{++} which the mitochondria can take up (their CC) without irreversible functional disturbances. To estimate CC the mitochondria were titrated with small quantities of $CaCl_2$ (100 nM at a time) until spontaneous release of Ca^{++}. The results of these experiments are given in Fig. 1. CC of mitochondria of the control animals was 55.4 ± 5.0 nmole H^+/mg protein, and in the liver 56.2 ± 3.9 nmole H^+/mg protein. Injection of adrenalin in a dose of 5 μg/100 g caused an increase of CC after 15 min in SIM by 25% (Fig. 1A, 1), whereas in the liver no change compared with the control was observed. The larger dose of adrenalin (25 μg/100 g) caused an increase of CC after 15 min by 38% in SIM, but by only 19% in the liver compared with the control, i.e., SIM mitochondria are more sensitive to the action of adrenalin. Accordingly, the effect of adrenalin on the rate of Ca^{++} uptake by SIM mitochondria also was investigated.

It will be clear from Fig. 2 that the smaller dose of adrenalin (5 μg/100 g) increased the rate of Ca^{++} uptake by 23%, the larger dose by 54%, compared with the control. The increase in the rate of inflow of Ca^{++} into

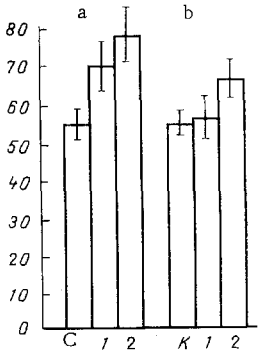

Fig. 1

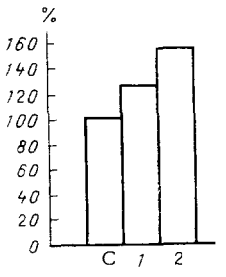

Fig. 2

Fig. 1. Effect of adrenalin on CC of mitochondria from rat SIM (A) and liver (B). Ordinate, CC of mitochondria (in nmole H^+/mg protein). 15 min before sacrifice animals received injection of physiological saline (C – control) or of adrenalin 5 μg/100 g (1) and 25 μg/100 g (2). Concentration of mitochondria in samples: for SIM 2.5 mg protein/ml, for liver 4.7 mg protein/ml.

Fig. 2. Stimulation of rate of Ca^{++} uptake by SIM mitochondria by adrenalin. Ordinate: rate of Ca^{++} uptake by mitochondria of control animals taken as 100%. Legend as to Fig. 1. Concentration of mitochondria in sample 2.4 mg protein/ml.

the mitochondria is directly related to the increase in their CC [13]. Another contributory factor may be stabilization of the mitochondria membrane by adrenalin, which other workers have observed [8, 11]. Activation of the rate of ATP synthesis by adrenalin may also stabilize the mitochondrial membrane [5]. The results are evidence of activation of energy functions in the initial period (15-30 min) of action of adrenalin both in liver mitochondria and in SIM. This direction of the reaction is in agreement with data obtained by other workers [3, 5, 8, 15]. However, the effect of adrenalin in stimulating oxidative phosphorylation and Ca^{++} uptake (especially in stress-inducing doses) is more marked in the mitochondria of SIM. Furthermore, whereas the activation of phosphorylation induced by injection of adrenalin was not observed in the liver mitochondria after 3 h, it was still present in the SIM mitochondria even after injection of adrenalin in a dose of 5 μg/100 g, but especially in a dose of 25 μg/100 g. This confirms previous conclusions that more sensitive, but poorly energized systems such as mitochondria of SIM compensate their structural-metabolic changes in response to various factors more slowly than the more resistant and highly energized systems such as liver mitochondria [7, 10].

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EFFECT OF ANTISYNAPTOSOMAL ANTIBODIES ON SYNAPTOSOMAL PROTEIN METABOLISM

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KEY WORDS: antibodies; brain; synapse; proteins; metabolism.

In recent years highly specific immunologic methods have been used on an increasingly wide scale to study mechanisms of brain function [1, 3, 4, 8, 9]. However, the molecular mechanisms of action of brain antibodies have not been adequately studied.

In the investigation described below the effect of intracerebral injection of antisynaptosomal antibodies on synaptosomal protein metabolism was studied.

EXPERIMENTAL METHOD

Rabbits were immunized by subcutaneous injection of a suspension of synaptosomes from rat cerebral cortex [6] in physiological saline (75 mg protein per animal in 2 ml of solution) with Freund's complete adjuvant. The animals were reimmunized 3 times with intervals of 1 month, without adjuvant. Blood was collected 7-12 days after each reimmunization. The antibody titer in the serum was determined by Ouchterlony's method of microprecipitation in agar [2]. The γ-globulin fraction was isolated by the method in [2].

The γ-globulin fraction for control experiments was isolated from nonimmune rabbit serum.

Experiments were carried out on male laboratory albino rats weighing 180 g, divided into three groups: control group 1) animals not receiving γ-globulin, control group 2) animals receiving nonimmune γ-globulin, experimental group 3) animals receiving injections of immune antisynaptosomal γ-globulin for 3 days (45 μl of dialysate in 1 min into the lateral ventricle).

Labeled precursor of protein synthesis, namely [^{14}C]protein digest of Chlorella (30 μl/min per animal) also was injected into the lateral ventricle of the rats. The animals were killed 2 h and 3 days after injection of the labeled precursor. In the first case the label was injected immediately after the antibodies, in the second case three daily injections of antibodies were given after the label. The synaptosomal fraction was isolated [6] from the brain of the decapitated animals and radioactivity counted in a dioxane scintillator system [5] on an Intertechnique SL-30 liquid scintillation counter. The significance of differences between the values was calculated by Student's test.

EXPERIMENTAL RESULTS

Values of specific radioactivity of synaptosomal proteins from the brain of the control animals and animals receiving nonimmune γ-globulin, when sacrificed 2 h and 3 days after injection of labeled Chlorella digest, were similar (Table 1). Meanwhile it was found that intraventricular injection of antisynaptosomal γ-globulin into the rats caused a significant increase in specific radioactivity of the proteins studied 2 h after, and a decrease in

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