CONTENT OF FREE NUCLEOTIDES IN SKELETAL MUSCLES AND HEART AFTER ADMINISTRATION OF HYDROCORTISONE

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The content of adenylnucleotides in the gastrocnemius muscles and heart of rabbits was investigated by electrophoresis on paper. Three hours after injection of hydrocortisone into rabbits (50 mg/kg) the content of ATP in the gastrocnemius muscles and heart was reduced, the content of AMP was increased, activity of cytochrome C-oxidase was lowered, and activity of lactate and succinate dehydrogenases was unchanged.

According to the literature, injection of hydrocortisone is followed by changes in the content of adenylnucleotides in the brain of animals [2, 3, 10, 11] and an increase in the level of carbohydrate and phosphorus metabolism in patients with rheumatic endarteritis and Addison's disease [4]. Glucocorticoids are known to modify the oxygen demand and activity of the respiratory enzymes of certain tissues [8, 9, 13, 14, 16, 18].

The object of the present investigation was to study the effect of hydrocortisone on the content of adenylnucleotides and the activities of cytochrome C-oxidase and succinate and lactate dehydrogenases.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits. In series I the animals received intramuscular injections of 3-4 ml of isotonic NaCl solution depending on body weight, and in series II they received 50 mg/kg body weight of a suspension of hydrocortisone. The dynamics of the changes in the plasma corticosteroid levels in experiments under these conditions have been described previously [10]. The rabbits were decapitated 3 h after injection of these substance, and the gastrocnemius muscle and heart were removed, frozen in liquid nitrogen, and ground into a powder. Creatine phosphate was determined in the powder as creatine (in mg%) by Delory's method in Grigor'eva's modification [6]. The content of adenylic acids was determined by electrophoresis on paper [5]. They were fractionated in 0.05 M citrate buffer (pH 4.8) at room temperature for 3.5 h in a voltage gradient of 8-10 V/cm. NF-1 chromatographic paper was used. After electrophoresis the paper strips were dried, developed in a chromatoscope, and the stains corresponding to ATP, ADP, and AMP were cut out and eluted in 5 ml 0.1 N HCl solution. Extinction was measured on a type SF-4A spectrophotometer at wavelengths of 260 and 290 nm in a cell with a layer thickness of 1 cm. The concentration was expressed in μmoles/g moist tissue, and the molar extinction coefficient was taken as 14,300.

Activity of the enzymes was determined in unfrozen pieces of tissue. The activity of cytochrome C-oxidase (1.9.3.1) was calculated in indophenol units (i.u.) /mg protein/min [20]. Activity of succinate dehydrogenase (1.3.99.1) was expressed in μg neotetrazan/mg protein [17]. Lactate-dehydrogenase activity (1.1.1.27) was investigated by Natelson's method and expressed in μg pyruvic acid/mg protein [12]. Protein in the samples was determined by Lowry's method [7]. The experimental results were analyzed by statistical methods.

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TABLE 1. Content of Adenylnucleotides (ATP, ADP, AMP – in μmoles/g moist tissue) and of Inorganic Phosphorus (P_i – in mg%), and Activity of Cytochrome C-Oxidase (CO – in indophenol units/mg protein/min), Succinate Dehydrogenase (SDH – in μg neotetrazan/mg protein), and Lactate Dehydrogenase (LDG – in μg pyruvic acid/mg protein) in Gastrocnemius Muscles and Heart of Rabbits after Injection of 50 mg/kg Hydrocortisone

Substance tested	Skeletal muscle		Heart muscle	
	control	expt.	control	expt.
ATP	5,50±0,31	4,29±0,33	2,74±0,29	1,34±0,16
ADP	1,09±0,22	1,25±0,13	1,51±0,10	1,30±0,09
AMP	0,82±0,07	0,20±0,09	0,76±0,053	0,93±0,057
Total	7,41	6,79	5,01	3,57
CO	$(39,0\pm3,7)\times10^{-3}$	$(26,5\pm3,25)\times10^{-3}$	$(261\pm17,1)\times10^{-3}$	$(194\pm11,0)\times10^{-3}$
SDH	14,9±2,5	12,8±1,88	708±56	605±60
LDH	1454±72	1394±81,9	1300±61,9	1208±17,8
P_i	64±2,9	67,3±5,33	37,5±2,2	46,5±2,22

EXPERIMENTAL RESULTS

According to P. and M. Feigelson [15], the metabolism of the peripheral tissues is changed 3-4 h after administration of corticosteroids. At the end of this period after administration of hydrocortisone in a dose of 50 mg/kg body weight to an animal, the blood plasma still contains a considerable quantity of corticosteroids, whereas 1 h after injection of smaller doses (2 mg/kg) the normal level is restored [10].

The results given in Table 1 show that the ATP content was significantly reduced in the skeletal muscles 3 h after injection of hydrocortisone. The ADP content in the muscle was slightly increased, while in the heart it was reduced. However, these decreases were not statistically significant. The AMP content in the skeletal and heart muscles was increased. The total content of nucleotides was reduced also. The ratio between the adenylic acids was altered. In the control experiments, for example, the ratio of ATP:ADP:AMP, expressed in percentages, was 74.4:14.7:10.9 in the skeletal muscles and 54.7:30.1:15.2 in the heart. This ratio 3 h after injection of 50 mg/kg hydrocortisone was 63.2:18.4:18.4 in the skeletal muscles and 37.5:36.4:26.1 in the heart. After injection of hydrocortisone, the ATP content was reduced and the AMP content increased. Similar changes after injection of the same dose of hydrocortisone have also been observed in the brain [2, 3]. The content of creatine phosphate in the gastrocnemius muscles was unchanged. Creatine phosphate could not be determined in the heart.

One reason for the decrease in the ATP level in the muscles and heart after injection of large doses of hydrocortisone could be inhibition of the activity of the respiratory chain enzymes, interfering with electron transfer and, hence, with ATP synthesis. Depression of electron transport in the respiratory chain after injection of large doses of hydrocortisone has been reported previously [8, 10, 19]. Hydrocortisone in a dose of 50 mg/kg depresses $NAD\cdot H_2$: cytochrome c-oxidoreductase activity in the brain [8, 9].

Simultaneous investigation of cytochrome oxidase activity and the content of adenylic acids in homogenates of the gastrocnemius muscles and heart in these experiments showed that 3 h after injection of 50 mg/kg hydrocortisone the ATP content was reduced, cytochrome c-oxidase activity was lowered, and the AMP content was raised (Table 1). Succinate dehydrogenase activity in the gastrocnemius muscles was reduced by 14%, and in the heart by 14.6%, while lactate dehydrogenase activity in the muscles was reduced by 5% and in the heart by 7%. The difference was not statistically significant. These enzymes are evidently more resistant to the action of hydrocortisone.

The investigations described above thus showed that hydrocortisone, in a dose of 50 mg/kg, reduced the ATP content and increased the AMP content in the gastrocnemius muscles and heart of rabbits. Cytochrome oxidase activity fell under these conditions. The creatine phosphate content was unchanged. The decrease in the ATP content was evidently due to disturbance of electron transport in the respiratory chain.

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