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EFFECT OF ACTH AND HYDROCORTISONE ON Ca^{++}-ATPase ACTIVITY OF THE SARCOPLASMIC RETICULUM OF SKELETAL MUSCLE

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The neuromuscular system and the pituitary–adrenocortical system are functionally interconnected. This connection is expressed in the fact that muscular activity modifies activity of the pituitary–adrenal system, and the hormones of the latter take part in adaptive reactions of the body to muscular activity [1, 2, 4]. ACTH and hydrocortisone have been shown to act through metabolic processes in the muscles, leading to changes in their contractile activity [3-6].

The Ca pump of the sarcoplasmic reticulum (SR), which regulates the intracellular Ca^{++} distribution, plays an important role in the mechanisms of development of muscular contraction. Meanwhile the mechanism of action of the adenohypophyseal and adrenocortical hormones on the work of this transport system of the myocytes has not yet been studied.

The aim of this investigation was to study the effect of ACTH and hydrocortisone on Ca^{++}-ATPase activity of SR of skeletal muscles.

TABLE 1. Ca^{++}-ATPase Activity (in μmoles P_i/min/mg protein) of SR Isolated from Gastrocnemius Muscles of Rats 1 h after Intraperitoneal Injection of ACTH (1 unit/100 g) and Hydrocortisone (5 mg/100g) (M ± m, n = 6)

Experimental conditions	ATPase activity of SR		
	total	basal	Ca^{++}-ATPase
Control	6,3±0,7	3,3±0,6	3,0±0,3
ACTH	8,5±0,1*	2,1±0,2	6,4±0,2*
Control	4,9±0,6	2,4±0,3	2,4±0,3
Hydrocortisone	8,9±0,8*	5,9±1,0*	3,0±0,4

Legend. Here and in Tables 2 and 3; *p < 0.05 compared with control.

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TABLE 2. Ca^{++}-ATPase Activity (in μmoles P_i/min/mg protein) of SR Isolated from Rabbit Skeletal Muscles, under the Influence of ACTH and Hydrocortisone in vitro (M ± m, n = 10)

Experimental conditions	ATPase activity of SR		
	total	basal	Ca^{++}-ATPase
Initial	7,1±0,5	1,4±0,1	5,7±0,5
ACTH, unit/ml			
0,05	8,2±0,5	0,7±0,1*	7,5±0,4*
0,005	11,1±0,8*	1,4±0,2	9,6±0,6*
0,0005	8,6±0,6	1,1±0,2	7,5±0,5*
Synacthen, units/ml			
0,05	8,2±0,5	1,2±0,2	7,1±0,7
0,005	12,5±0,8*	1,3±0,2	11,2±1,0*
0,0005	8,9±0,4*	1,3±0,1	7,6±0,5*
Hydrocortisone, M			
$2,8 \cdot 10^{-6}$	6,3±0,1	1,1±0,1	5,2±0,18
$2,8 \cdot 10^{-7}$	10,2±0,6*	1,2±0,1	9,0±0,7*
$2,8 \cdot 10^{-8}$	8,7±0,6	1,0±0,1	7,6±0,6*

TABLE 3. Accumulation of Calcium and Ca^{++}-ATPase (in nmoles/min/mg protein) in SR Vesicles Isolated from Rabbit Skeletal Muscles (M ± m, n = 8)

Experimental conditions	Ca^{++}-accumulation	Ca^{++}-ATPase	Ca/ATP
Control	674,1±20,4	1495,2±133,8	0,5±0,03
ACTH, unit/ml			
0,05	747,0±25,1*	1634,0±114,6	0,5±0,03
0,005	993,4±55,0*	1589,5±133,6	0,6±0,04*
0,0005	1575,5±114,9*	1605,0±233,3	1,0±0,1*
0,00005	765,4±21,0*	1415,0±145,3	0,6±0,06
Control	596,0±32,5	1235,0±54,4	0,5±0,03
Synacthen, units/ml			
0,05	667,0±24,3	1666,0±85,9*	0,4±0,02*
0,005	1125,4±29,9*	1769,0±97,4*	0,6±0,02*
0,0005	1672,0±70,6*	1835,6±111,4*	0,9±0,05*
0,00005	827,0±19,0*	1292,0±58,4	0,6±0,02*
Control	596,0±32,5	1235,2±54,5	0,5±0,03
Hydrocortisone, M			
$2,8 \cdot 10^{-6}$	466,0±15,8	2289,0±154,4*	0,2±0,02*
$2,8 \cdot 10^{-7}$	784,0±15,5*	2221,0±80,7*	0,4±0,01*
$2,8 \cdot 10^{-8}$	769,0±35,3*	2082,0±77,4*	0,4±0,02*
$2,8 \cdot 10^{-9}$	967,0±58,2*	1674,0±162,1*	0,6±0,03*

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 190-200 g, which were given an intraperitoneal injection of $ACTH_{1-39}$ in a dose of 1 unit/100 g and of hydrocortisone hemisuccinate in a dose of 5 mg/100 g body weight. Animals of the control group received an injection of isotonic NaCl solution. The rats were decapitated 1 h after injection of the hormones. SR was isolated from the gastronemius muscles [7] and its Ca^{++}-ATPase determined.

The effect of $ACTH_{1-39}$, Synacthen ($ACTH_{1-24}$), and hydrocortisone hemisuccinate on the Ca^{++}-ATPase of SR, isolated from rabbit skeletal muscles, on Ca^{++} accumulation in the vesicles of SR, and on their Ca^{++}-ATPase activity was studied in experiments in vitro.

Ca^{++}-ATPase activity was determined as the difference between Ca,Mg-ATPase (total) and Mg^{++}-ATPase (basal) activity in relation to the quantity of inorganic phosphorus (P_i) removed from the substrate in 1 min at 37°C [11]. Phosphorus was determined by the method in [18] and protein by Lowry's method [10].

Accumulation of Ca^{++} in SR vesicles was determined by adding the radioactive label $^{45}CaCl_2$ to the incubation mixture [11]. Radioactivity was measured with an SL-4000 counter (France). The Ca^{++}-ATPase activity of the vesicles was determined in the same samples as the rate of removal of phosphorus. $ACTH_{1-39}$ and $ACTH_{1-24}$ were added to the incubation medium up to a final concentration of 0.05-0.00005 unit/ml, and hydrocortisone hemisuccinate up to a concentration of $2.8 \cdot 10^{-6}$-$2.8 \cdot 10^{-9}$ M.

EXPERIMENTAL RESULTS

Table 1 shows that after injection of $ACTH_{1-39}$ into the rats the total ATPase activity of SR of the gastrocnemius muscles rose by 34%, Ca^{++}-ATPase activity rose by 113%, whereas Mg^{++}-ATPase activity fell by 35%. After injection of hydrocortisone into the rats their total ATPase activity of SR rose by 81%, Mg^{++}-ATPase rose by 140%, and Ca^{++}-ATPase activity was unchanged.

Experiments in vitro with Ca^{++}-ATPase preparations isolated from SR of rabbit skeletal muscles showed that $ACTH_{1-39}$ and $ACTH_{1-24}$ in concentrations of 0.05 to 0.00005 unit/ml increased the Ca^{++}-activated ATPase activity but caused no change in Mg^{++}-activated ATPase activity.

Hydrocortisone in concentrations of $2.8 \cdot 10^{-7}$ to $2.8 \cdot 10^{-8}$ M also increased Ca^{++}-ATPase activity (Table 2). Hydrocortisone is known to increase binding of ^{45}Ca by rat liver membranes also [9]. The increase in Ca^{++}-ATPase activity under the influence of $ACTH_{1-39}$ and $ACTH_{1-24}$ is in agreement with experimental results showing that these hormones increase the Ca^{++} content in SR vesicles and the Ca/ATP ratio. Hydrocortisone in a concentration of $2.8 \cdot 10^{-7}$ to $2.8 \cdot 10^{-9}$ M also increased Ca^{++} accumulation in SR vesicles, increased Ca^{++}-ATPase activity, but lowered the Ca/ATP ratio. However, in a concentration of $2.8 \cdot 10^{-6}$ M, hydrocortisone reduced Ca^{++} accumulation and lowered the Ca/ATP ratio, while at the same time increasing the Ca^{++}-ATPase of these vesicles (Table 3).

These investigations thus showed that $ACTH_{1-39}$ and $ACTH_{1-24}$ increase Ca^{++}-ATPase activity, Ca^{++} accumulation in the vesicles of SR, and the Ca/ATP ratio. Hydrocortisone, on the other hand, increases Ca^{++}-ATPase activity and Ca^{++} accumulation in the vesicles of SR, but lowers the Ca/ATP ratio. These facts are evidence that ACTH stimulates Ca^{++} accumulation in SR with lower consumption of the energy of ATP. Hydrocortizone, on the other hand, stimulates Ca^{++} accumulation in the SR vesicles with a higher consumption of the energy of ATP. We obtained similar results also in a study of the effect of ACTH and hydrocortisone on the creatine phosphate content of rabbit muscles, which showed that ACTH reduces, whereas hydrocortisone increases creatine phosphate breakdown per unit work of the muscle [4].

The changes described above may be the result of the effect of the hormones on membrane permeability of SR and direct interaction with ATPase. In fact, ACTH and hydrocortisone both increase the Ca^{++}-ATPase activity of SR isolated from skeletal muscles in experiments in vitro also.

The existence of a similar mechanism of action of these two hormones on Ca^{++} transport in skeletal muscles can also be postulated in the intact organism, provided that hydrocortisone can pass freely through the cell membrane, and that ACTH can "pierce" the phospholipid layer of the membrane and can interact with the intracellular components by one end of its polypeptide chain [12, 13].

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