biosynthesis and, consequently, to fresh release of PG. It can be expected that minimal GP activity will correspond to the maximal PG level.

GT is the key enzyme of biodegradation of epoxides in the small intestine and one of the principal enzymes of DES in the liver. The sharp decline in its activity is evidence of severe damage to the antitoxic system of the small intestinal mucosa.

We know that CT does not penetrate into the blood stream [7, 15] and, as the writers' experiments *in vitro* have shown, it does not affect enzymes of DES (data not published in this paper). It can be concluded that the general character of changes in enzyme levels in the liver and mucosa is determined by the indirect action of the toxin on the enzymes concerned. Most probably the cause of the changes observed in DES is excessive formation of toxic substances, namely inhibitors of the enzymes indicated above or the systems for their biosynthesis, in this experimental model of a pathological process. Causes of the increase in activity in the second stage are even less clear and may be connected with a compensatory reaction of the body leading to activation of apoenzymes and stimulation of their synthesis. The possibility of an increase in contamination with erythrocytic enzymes, on account of microcirculatory disturbances in the intestinal mucosa, likewise cannot be ruled out.

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EFFECT OF ACTH AND HYDROCORTISONE ON cAMP CONTENT AND PHOSPHODIESTERASE ACTIVITY IN RAT SKELETAL AND SMOOTH MUSCLES

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Adrenocortical hormones are known to have a significant effect on various types of metabolism in muscle tissue [1, 4]. However, the mechanisms of this influence have not yet been explained. It can be postulated that their action on muscles is realized through the adenylate cyclase system. For instance, investigations have shown that ACTH and corticosteroids modify the cyclic AMP (cAMP) content in some organs and tissues [3, 7, 14, 15], and cAMP helps to control activity of the enzymes of glycolysis and the respiratory chain, and of transport ATPases [2, 5, 9, 11-13, 15].

The aim of this investigation was to study the effect of ACTH and hydrocortisone on the cAMP content and cyclic nucleotide phosphodiesterase (PDE) activity in rat skeletal and smooth muscles.

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EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-200 g. The animals were decapitated and the gastrocnemius muscle and muscle from the fundus of the stomach were removed. The tissue was frozen in nitrogen, ground to a powder, and treated with absolute ethanol in the ratio of 1:10. The cAMP concentration was determined by competitive radioisotope binding [10], using standard kits from Amersham Corporation (England). To study PDE activity, the isolated muscle tissue was homogenized with 0.5 M Tris-HCl, pH 8.2. The homogenate was centrifuged at 2000g for 15 min, and PDE activity was determined in the supernatant by a radiometric method using kits from the same firm [8]. Protein in the samples was determined by Lowry's method. The plasma 11-hydroxycorticosteroid (11-HCS) level was determined by the standard fluorometric method [8]. ACTH (from Kaunas Endocrine Preparations Factory), in doses of 1 and 2 units/100 g body weight, and hydrocortisone (from Gedeon Richter, Hungary), in doses of 1 and 5 mg/100 g body weight, were injected intraperitoneally. Physiological saline was injected into the control animals in a dose of 0.5 ml/100 g body weight.

The cAMP content and PDE activity in the muscles and the plasma 11-HCS level were determined 1 and 3 h after injection of the preparation. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The data showed (Table 1) that the cAMP content in the skeletal and smooth muscles of the control rats did not differ significantly. PDE activity was more than 1.5 times higher in smooth muscle than in skeletal. The cAMP content in the gastrocnemius muscle 1 h after injection of ACTH in a dose 1 unit/100 g was increased by 31.6% ($P < 0.05$), and after injection of hydrocortisone in a dose of 1 mg/100 g, it was increased by 34.6% ($P < 0.02$). A greater increase in the cAMP content in the gastrocnemius muscle was observed 3 h after injection of two units/100 g of ACTH and 5 mg/100 g of hydrocortisone. For instance, the cAMP content after injection of ACTH was increased by 86.03% ($P < 0.001$), and after injection of hydrocortisone by 78.5% ($P < 0.001$).

The cAMP content in smooth muscle 1 h after injection of 1 unit/100 g ACTH was increased by 33.25% ($P < 0.001$), and after injection of 1 mg/100 g hydrocortisone it was increased by 18.55% ($P > 0.05$). The cAMP content 3 h after injection of two units/100 g of ACTH was increased by 64.01% ($P < 0.001$), and after injection of 5 mg/100 g of hydrocortisone by 58.17% ($P < 0.001$).

The increase in the cAMP content in the tissues could be connected either with increased adenylate cyclase activity or with reduced PDE activity.

Experiments showed that 1 h after injection of 1 unit/100 g of ACTH, PDE activity in the muscles was unchanged, whereas after injection of 1 mg/100 g of hydrocortisone its activity was depressed in skeletal muscle by 29.11% ($P < 0.05$), and in smooth muscle by 25.2% ($P < 0.05$).

These facts are evidence that the increase in the cAMP content in response to injection of ACTH was the result of adenylate cyclase activation, whereas reduction of PDE activity could play a significant role in the increase in the cAMP content after injection of hydro-

TABLE 1. 11-HCS Concentration in Blood Plasma (in μg/100 ml plasma) and cAMP Content in pmoles/g tissue) and PDE Activity (in pmoles cAMP/min/mg protein) in Gastrocnemius Muscle and Gastric Muscle of Rats after Injection of ACTH and Hydrocortosone (M ± m; n = 7 or 8)

Substance injected	Time after injection, h	11-HCS	cAMP		PDE	
			skeletal muscle	smooth muscle	skeletal muscle	smooth muscle
Physiological saline	1	20,05±2,30	352,60±18,15	311,60±14,20	0,79±0,05	1,18±0,06
ACTH, 1 unit/100 g	1	30,85±4,06*	464,00±29,00	415,00±9,40*	0,69±0,05	1,07±0,06
Hydrocortisone, 1 mg/100 g	1	38,65±4,53*	474,60±28,40*	369,40±18,6	0,56±0,05*	0,88±0,08*
Physiological saline	3	17,50±3,45	345,80±18,10	314,60±15,70	0,80±0,05	1,18±0,06
ACTH, 1 unit/100 g	3	57,78±7,07*	643,30±19,97*	516,00±18,90*	0,48±0,07*	0,51±0,08*
Hydrocortisone, 5 mg/100 g	3	45,12±3,63	617,25±29,71	497,60±16,40	0,50±0,08	0,64±0,08

Legend. *$P < 0.05$ compared with corresponding control.

cortisone. This last suggestion also was confirmed by the coefficients of correlation between the plasma 11-HCS level and the cAMP content in the gastrocnemius muscle. For instance, 1 h after injection of 1 unit/100 g ACTH no such correlation was present, whereas after injection of 1 unit/100 g of hydrocortisone, moderately strong correlation was found with a coefficient of 0.668 ($P < 0.05$). The increase in the cAMP content in the muscles in the early stages after injection of ACTH was thus independent of the plasma 11-HCS level. However, 3 h after injection of 2 units/100 g of ACTH, PDE activity was depressed in the gastrocnemius and smooth muscles by 40% ($P < 0.01$) and by 56.8% ($P < 0.001$) respectively. After injection of 5 mg/100 g of hydrocortisone PDE activity was depressed at these times by 37.5% in the gastrocnemius muscle ($P < 0.001$) and by 45.8% in the smooth muscle ($P < 0.001$).

The rise in the cAMP content 3 h after injection of ACTH was linked with a raised plasma 11-HCS level and inhibition of PDE activity.

Investigation of the plasma 11-HCS levels of these rats showed that 1 h after injection of 1 unit/100 g of ACTH they were raised by about 1.5 times, whereas after injection of 1 mg/100 g of hydrocortisone they were approximately doubled. The 11-HCS concentration 3 h after injection of 2 units/100 g of ACTH was increased by 3.3 times, and after injection of 5 mg/100 g of hydrocortisone, by 2.57 times.

Under these experimental conditions, both ACTH and hydrocortisone thus induce corresponding changes in the blood 11-HCS level, in full agreement with observed changes in PDE activity and cAMP content in muscle tissue. The increase in the cAMP content in the early stages after injection of ACTH, accompanied by a very small rise in the blood 11-HCS level was not connected with changes in PDE activity and, consequently, it was the result of adenylate cyclase activation. The increase in the cAMP content in the skeletal and smooth muscles in response to ACTH injection is linked initially with adenylate cyclase activation, but later, with an increase in the release of corticosteroids, which inhibit cAMP hydrolysis, into the blood stream. Phosphodiesterase activity is inhibited by corticosteroids by a greater degree in smooth muscles than in skeletal muscles.

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