To study the specificity of the activating effect of o,p'-DDD experiments were carried out with the cytoplasmic fraction of the liver and kidneys (Table 2).

The results are evidence that o,p'-DDD activates the enzyme only in the adrenal. Similar results were obtained by the writers when feeding dogs with o,p'-DDD. These observations confirm the high specificity of action of the inhibitor on the adrenal cortex.

It can be postulated on the basis of these results that the marked activation of the enzyme by o,p'-DDD and Perthane in vitro is a result of the direct interaction between the diphenyldichloroethane derivatives and glutathione reductase, but the mechanism of this activation is not yet clear.

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FREE FATTY ACID CONTENT IN MUSCLES AFTER ADMINISTRATION OF ACTH AND HYDROCORTISONE

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The content of free fatty acids (FFA) in the gastrocnemius muscles 30 min after intraperitoneal injection of 1 unit ACTH/100 g and 1 mg hydrocortisone acetate/100 g body weight was investigated by gas chromatography in experiments on rats. In resting muscles ACTH was shown to increase the stearic acid content whereas hydrocortisone increased the content of both stearic and oleic acids. Changes in the concentration of other FFA were not significant. During a short period of activity involving single regular contractions the stearic acid concentration in the gastrocnemius muscles of intact rats increased. In the experiments with ACTH and hydrocortisone this increase was considerably smaller and was not significant. ACTH and hydrocortisone stimulate the utilization of stearic acid by the muscles during activity.

KEY WORDS: ACTH, hydrocortisone, free fatty acids, muscular contraction.

Hormones of the adenohypophysis and adrenal cortex play a special role in the regulation of lipid metabolism, for significant disturbances of this type of metabolism are observed when they are deficient. However, data on the role of these hormones in the regulation of lipid metabolism are to some degree contradictory. Some workers, for instance, found a decrease in the neutral lipid content in the liver and blood plasma of animals after adrenalectomy [14], whereas others found no such changes [12]. On the other hand, stress has been shown to increase the blood level of free fatty acids (FFA) while at the same time increasing the lipolytic activity of the adipose tissue and liver [7].

Existing data on the effect of ACTH and glucocorticoids on lipolysis are concerned chiefly with adipose tissue and the liver. However, unequivocal data have been obtained only for ACTH. Injection of preparations of this hormone stimulates lipolysis of the adipose tissue and increases the fatty acid concentration in blood plasma [1, 4, 5]. There are few data on glucocorticoids and they point to absence of any effect on lipolysis

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TABLE 1. Effect of ACTH and Hydrocortisone on Free Fatty Acid Content (in μg/g tissue) of Gastrocnemius Muscles of Rats during Activity (M ± m)

Experimental conditions / FFA	Control (10)		ACTH (7)		Hydrocortisone (7)	
	rest	activity	rest	activity	rest	activity
Myristic	0,300±0,059	0,410±0,160	0,570±0,240	0,257±0,088	0,500±0,230	0,600±0,180
Palmitic	22,86±0,65	19,8±0,43*	22,7±0,30	22,76±0,10	23,14±0,74	21,45±0,65
Stearic	14,90±2,14	33,70±8,00*	25,20±2,75*	33,80±5,29	26,40±4,40*	29,30±4,99
Oleic	7,70±1,95	10,50±2,06	13,80±4,38	11,30±4,54	17,20±2,60*	20,80±3,60
Linoleic	7,60±3,06	3,70±0,45	7,00±3,18	9,32±3,48	3,80±0,78	0
Arachidonic	3,60±0,71	2,28±0,38	3,04±0,35	3,92±0,74	2,70±0,73	0

Legend. Number of experiments shown in parentheses; asterisk denotes significance of difference with corresponding control ($P < 0.05$).

of adipose tissue [5, 11]. The effect of ACTH and glucocorticoids on lipid metabolism in other tissues has received even less study, and virtually none in skeletal muscles.

It was accordingly decided to study the effect of ACTH and hydrocortisone on the FFA concentration in muscles in various functional states.

EXPERIMENTAL METHOD

Male rats weighing 220-250 g were used. Under urethane anesthesia the tibial nerves and Achilles' tendons were dissected on both sides. Platinum stimulating electrodes were applied to the right tibial nerve. The Achilles' tendons were connected to a myograph to record contractions of the muscle. Muscular contraction was isotonic against a load of 20 g. Contraction was induced by stimulation of the nerve with square pulses of above-threshold strength, 1.5 msec in duration and with a frequency of 2 Hz for 5 min. The hormone level in the body was varied by injection of exogenous preparations of ACTH and a suspension of hydrocortisone acetate (from Gedeon Richter, Hungary). Stimulation began 30 min after intraperitoneal injection of 0.2 ml physiological saline/100 g body weight (series I); 1 unit ACTH/100 g body weight (series II), and 1 mg hydrocortisone acetate/100 g body weight (series III). Both muscles were removed and homogenized after the end of stimulation. The left muscle, which was not stimulated, served as the control. Lipids were extracted with ether. The fatty acids were methylated with diazomethane [9]. The methyl esters of the FFA were fractionated in a Tsvet-100 gas chromatograph on a glass column packed with 1% SE-30; the size of the grains was 0.16-0.20 mm. The column temperature could be programmed between 100 and 230°C. Hydrogen was used as the carrier gas. The sample was introduced in a volume of 1-5 μl. The analysis continued for 30 min and ended after elution of the arachidonic acid. The concentrations of individual fatty acids were calculated by comparing the area of the peaks obtained in the experimental series with the area of the corresponding peaks of known quantities of standard methyl esters of fatty acids and expressed in μg/g tissue.

EXPERIMENTAL RESULTS

The experiments showed that the contractile power of the muscles, measured at the height of contraction, was reduced by 20% in the control rats, by 23% in the experiments with ACTH, and by 8.6% after injection of hydrocortisone relative to the maximal shortening of the muscle observed during the period of stable working capacity. The impression was obtained that hydrocortisone reduces muscle fatigue. However, in these experiments the contractile power of the muscle was lower than in the control series or after injection of ACTH. Further evidence was given by the quantity of work done by the muscle in 5 min. In the control experiments it was $(788 \pm 110) \times 10^{-5}$ kg·m; after ACTH it was $(735 \pm 25) \times 10^{-5}$ kg·m, and after injection of hydrocortisone $(419 \pm 44) \times 10^{-5}$ kg·m, or 46.8% less than in the control.

Investigation of the FFA content showed that the gastrocnemius muscles of the rats at rest contained (in all experiments, in higher concentrations than other components) palmitic, stearic, and oleic acids. Myristic, linoleic, and arachidonic acids were not found in some of the experiments.

A tendency toward an increase in the concentrations of myristic and oleic acids was observed 30 min after injection of ACTH. The stearic acid content was increased by 10.3 μg/g tissue. After injection of hydrocortisone the concentration of stearic acid increased significantly by 11.5 μg/g and that of oleic acid by 9.5 μg/g. Changes in the concentrations of the other acids were not significant (Table 1).

Investigation of the concentrations of these acids in the muscles during activity showed an increase of 18.8 μg/g in the stearic acid concentration and a decrease of 3.06 ± 0.57 μg/g tissue of palmitic acid in the control rats. After injection of ACTH or hydrocortisone the increase in the stearic acid concentration was reduced by 73 and 92% respectively, whereas the concentration of palmitic acid was unchanged (Table 1). In the experiments with hydrocortisone linoleic and arachidonic acids were not determined during activity.

Clearly 30 min after injection of ACTH or hydrocortisone the concentrations of individual FFA in the muscles were increased. The increase can be explained by the effect of these hormones on lipolysis [1, 4-6, 13, 15].

The mechanism of action of ACTH on lipolysis in muscle tissue is evidently the same as in adipose tissue and is connected with its influence on the cyclic AMP level [10]. Data showing that a cyclic AMP-dependent protein kinase phosphorylates and activates ACTH-sensitive phospholipase relative to triolein [13] may also be confirmation of this mechanism.

The mechanism of action of hydrocortisone may be somewhat different, for it penetrates freely into the cell and can exert its influence on the activity of enzymes, including lipase, so as on the one hand to modify the synthesis of new enzyme protein and, on the other hand, to change the activity of enzyme already synthesized, by a form of allosteric regulation [3].

Since an increase in the FFA concentration in the muscles was observed as early as 30 min after intraperitoneal injection of the hormone, it can be tentatively suggested that in this case hydrocortisone influences the conformation of the lipolytic enzymes.

The increase in the stearic acid concentration in the control muscles during activity may have been due, on the one hand, to an increase in its supply from the fat depots on account of the increased blood flow, and on the other hand, to increased lipolysis in the muscle tissue. During activity of short duration, FFA in the muscles can be assumed to undergo negligible oxidation. The level of nonesterified fatty acids in the blood serum of rats is known [8] to be only very slightly reduced after swimming for 15 min.

In the experiments with ACTH, during activity involving insignificant FFA utilization, the FFA concentration in the muscles should evidently have increased even more than in the control. It will be recalled that muscular exertion raises the blood ACTH level [2]. In reality the opposite response was observed: not an increase but a decrease of 83% in the stearic acid concentration compared with the control. Presumably ACTH stimulates the utilization of FFA by the muscles during activity.

The effect of hydrocortisone on stearic acid utilization by the muscles during activity was more marked. This can be seen more clearly still if it is remembered that the work done by these muscles was only 53% of that done by the control muscles.

These investigations thus showed that administration of ACTH and hydrocortisone increases the concentration of saturated FFA and, in particular, of stearic acid, in the muscles. During activity of short duration involving regular contractions, the content of stearic acid in the muscles of the control animals increased whereas the palmitic acid level fell. ACTH and hydrocortisone stimulate the utilization of stearic acid during activity.

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