sponses of ablation of the labyrinth: stepping movements, rotation and tilting of the head toward the side of the destroyed labyrinth, extension of the left forelimb, deviation and nystagmus of the eyes. The intensity of these responses and the dynamics of their extinction were indistinguishable from those in labyrinthectomized animals with an intact spinal cord. A pattern similar to that described above was observed in the animals of group 2, in which the spinal cord was divided at the same time as labyrinthectomy (Figs. 1 and 2). However, division of the spinal cord at various times after labyrinthectomy caused no signs of disturbances of compensation in any of the animals: nystagmus and tilting the head were absent, rotation of the head and the position of the forelimbs remained unchanged. Subsequent inhalation of ether or chloroform by these animals, however, led to the development of the typical picture of decompensation. Inhalation of ether and chloroform by guinea pigs with intact labyrinth and spinal cord revealed no changes resembling the pattern of unilateral labyrinthectomy.

The experiments showed that spinal afferentation from the lower half of the body has no appreciable effect on compensation of the sequelae of unilateral loss of vestibular function and decompensation due to inhalation of general anesthetics. The results thus show that the disturbance of compensation in unilaterally labyrinthectomized guinea pigs in Azzena's experiments [3] was not the result of removal of spinal afferentation from the lower half of the body, but was an artifact caused by division of the spinal cord under ether anesthesia.

Under the influence of inhalational anesthetics decompensation probably arises as a result of depression of electrical activity of the vestibular nuclei on the side of labyrinthectomy, which disappears after destruction of the labyrinth but is restored in the course of compensation. This pehnomenon, in turn, leads to an imbalance of activity between the vestibular nuclei on the two sides, and, consequently to the reappearance of the extinguished responses [1]. It is not quite clear how ether and chloroform block the processes maintaining activity of the deafferented vestibular nuclei. One way may perhaps be through the inhibitory effect of these substances on the activity of certain brain enzymes [4].

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INCREASED SENSITIVITY OF ADIPOSE TISSUE OF SPONTANEOUSLY HYPERTENSIVE RATS TO ACTH. THE ROLE OF CALCIUM

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The action of ACTH on lipolysis was studied in the adipose tissue of rats with spontaneous and renal hypertension and also in normotensive rats of corresponding control groups. The sensitivity of the adipose tissue of SHR rats to ACTH was shown to be higher than in the normotensive control. Evidence was obtained that this increase in sensitivity is due to the state or quantity of intracellular calcium. In rats with renal hypertension no such increase in sensitivity of their adipose tissue to ACTH was found.

KEY WORDS: hypertension; ACTH; lipolysis; calcium.

Changes in the response of the adipose tissue to insulin and adrenalin have been found in rats with spontaneous and renal hypertension compared with their normotensive control [2,

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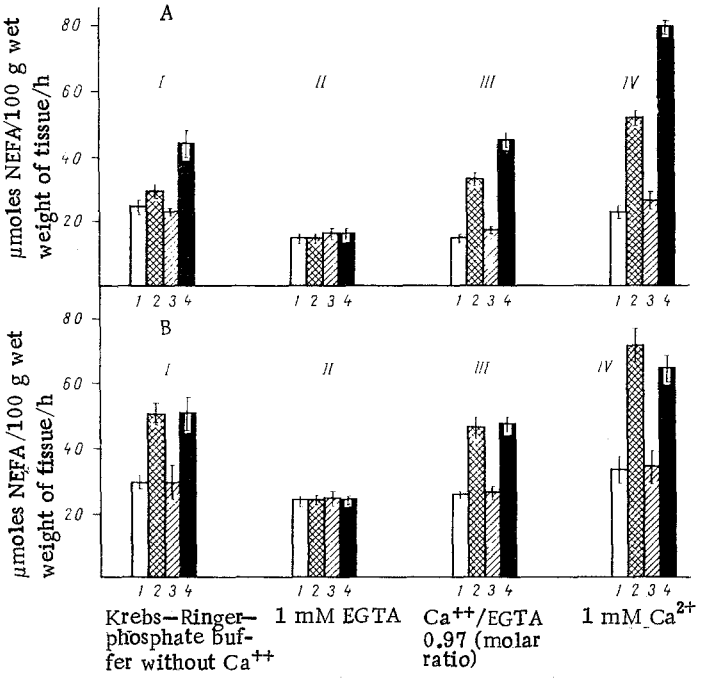

Fig. 1. Action of ACTH on NEFA synthesis in adipose tissue of hypertensive and normotensive rats under different conditions of incubation. Each result is mean of eight experiments. 1, 2) Normotensive, 3, 4) hypertensive rats; 1, 3) without ACTH; 2, 4) with ACTH. A) Spontaneous genetic hypertension; B) renal hypertension.

3]. The differences observed were regarded as evidence of a change in the function of the plasma membrane of the adipose cells in hypertensive animals, and the hypertrophy of the adrenal cortex and stimulation of corticosteroid secretion [1, 6] were regarded as a possible measure of the compensation of these disturbances. One regulator of the level of corticosteroid secretion by the adrenals in ACTH, sensitivity to which can be judged from the degree of its lipolytic action on adipose tissue. In this investigation this lipolytic action of ACTH was studied on the adipose tissue of rats with spontaneous and renal hypertension, and since the action of ACTH on its cell "target" is very closely connected with the presence of Ca^{++}, the dependence of the action of ACTH on the calcium ion concentration in the incubation medium also was studied.

EXPERIMENTAL METHOD

The following animals were used in two series of experiments: inbred male SHR (Kyoto–Wistar spontaneously hypertensive rats) in series I, and rats with renal hypertension in series II. The method of producing renal hypertension and the characteristics of the hypertensive animals and their controls were given in [2].

The rats were decapitated after deprivation of food for 24 h but with free access to water; the epididymal adipose tissue was then removed and the thin distal part (80-100 mg) was cut off and placed in incubation medium without ACTH (adipose tissue from the right epididymis) and with ACTH (tissue from the left epididymis). The ACTH concentration in samples producing the maximal lipolytic effect in all species of animals tested was 1 μg/ml (ACTH, 93 units/mg, from Serva, West Germany).

Four variants of experiments were carried out in order to study the dependence of the lipolytic action of ACTH on adipose tissue on the Ca^{++} ion concentration.

In variant I the adipose tissue was preincubated in 2 ml calcium-free Krebs–Ringer–phosphate (KRP) buffer, pH 7.4 [5], at 37°C with constant shaking for 1 h, after which ACTH was added to half of the samples and incubation of all samples continued for a further 1 h. At the end of incubation the samples were placed in ice and the concentration of nonesterified

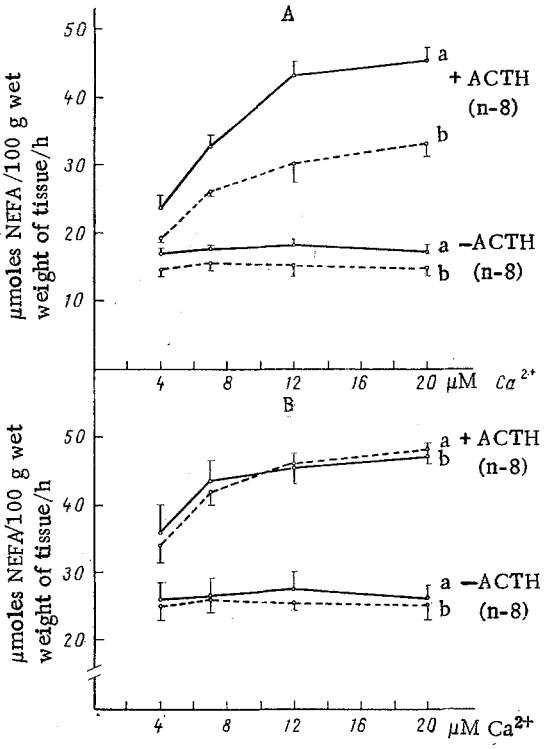

Fig. 2. Dependence of lipolytic action of ACTH in adipose tissue of hypertensive (a) and normotensive control rats (b) on free Ca^{++} concentration. A, B) As in Fig. 1.

fatty acids (NEFA) was determined by the method in [7]. An aliquot (0.1-0.2 ml) was taken from the incubation sample and the glycerol concentration in it determined by an enzymatic method [8].

In variant II the adipose tissue was preincubated in calcium-free KRP buffer with the addition of 1 mM EGTA (from Sigma, USA); the conditions of preincubation and of subsequent incubation with the hormone were the same as in variant I.

In variant III the adipose tissue was preincubated in calcium-free KRP buffer with the addition of 1 mM EGTA and Ca^{++} ions. The free calcium concentration was calculated on the assumption that the stability constant of the complex was $10^{6 \cdot 2}$. The molar proportions of Ca/EGTA were 0.86, 0.92, 0.95, and 0.97, i.e., the free Ca^{++} concentration was 4, 7, 12, and 20 μM respectively. The subsequent procedures were as in variant I.

In variant IV the tissue was preincubated in KRP buffer with a Ca^{++} concentration in the medium of 1 mM. The subsequent procedures were as in variant I.

Since the results for both lipolytic reactions were identical, only data on NEFA synthesis in the tissue are given in Figs. 1 and 2.

EXPERIMENTAL RESULTS AND DISCUSSION

Spontaneous Hypertension. The lipolytic action of ACTH on adipose tissue was about 4 times greater than its action on the adipose tissue of normotensive animals in calcium-free KRP buffer and almost twice as high in KRP buffer with 1 mM Ca^{++} (Fig. 1A, I, IV). The action of ACTH on lipolysis is known to require the presence of Ca^{++} ions. The difference between the sensitivity of adipose tissue of normotensive and hypertensive animals to ACTH may be due to the quantity of calcium bound with the plasma membranes of the cells (which is greater in the second case), or to different binding constants of the cations for the same number of binding centers. In both cases Ca^{++} adsorption must depend on the phospholipid composition [4] and structural state of the membranes. Accordingly, dependence of the effect of ACTH on the adipose tissue on the free Ca^{++} concentration in the incubation medium was investigated. In both groups of animals, when the adipose tissue was incubated with EGTA (1 mM) sensitivity

to ACTH was completely lost (Fig. 1A, II). The EGTA removed mainly the intercellular calcium and the calcium bound with the outer surface of the membranes from the tissue. The presence of free calcium in the incubation medium, in increasing molar proportions with EGTA, resulted in stimulation of lipolysis in the presence of ACTH (Fig. 1A, III; Fig. 2). It will be clear from Fig. 2 that the sensitivity of the adipose tissue of the hypertensive animals to ACTH was 1.5 times greater than that of the normotensive animals, for all the above-mentioned concentrations of free Ca^{++}. Because of the constancy of this ratio several suggestions can be made regarding the character of interaction between Ca^{++} ions and the cell membranes of hypertensive and normotensive animals. In the first case the cell membranes may perhaps bind 1.5 times more Ca^{++} because of an increase in the apparent affinity constant of the adsorption centers for cations or because of an increase in their number. In both cases changes in calcium binding could potentiate the postreceptor signal of ACTH on the membrane. Furthermore, the passive permeability of the membranes for Ca^{++} may be increased by 1.5 times in the hypertensive animals, and an increase in the intracellular Ca^{++} concentration would lead to activation of certain intracellular processes and, in particular, of lipolysis. On the other hand, an increase in the intracellular Ca^{++} concentration in the hypertensive animals may be due to a decrease in the efficiency of the active calcium removal system (the Ca pump). All the alternative suggestions explaining differences in the sensitivity of adipose tissue to the calcium-dependent action of ACTH are evidently based on changes in the protein-lipid composition determining the state of the membranes and their sensitivity to hormones.

Renal Hypertension. In the rats with experimental renal hypertension no difference could be found in the sensitivity of the adipose tissue of the hypertensive and control rats to ACTH (Fig. 1B, 2). In this case the cell membranes of the two groups of animals evidently had identical ability to bind calcium and, consequently, for ACTH to interact with the plasma membranes of the cells.

The results thus indicate that genetically determined differences in biosynthesis and, consequently, in the protein-lipid composition of the membranes of adipose cells, determine differences either in the degree of binding or in the magnitude of Ca^{++} transport in hypertensive and normotensive animals, and that this, in turn, is responsible for the difference in sensitivity of these tissues to ACTH.

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