STRUCTURAL DIFFERENCES BETWEEN ADRENALIN AND ACTH RECEPTORS IN RAT ADIPOCYTES

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Treatment of adipose tissue from the rat epididymis with trypsin reduced the lipolytic action of ACTH by 95% and the lipolytic action of adrenalin by 50%. Addition of lysolecithin to the incubation sample led to total loss of sensitivity of the tissue to ACTH and reduced its sensitivity to adrenalin by 40%. It is concluded that there are structural differences between adrenoreceptors and ACTH receptors in the plasma membrane of adipocytes.

KEY WORDS: adipose tissue; adrenalin; ACTH; lipolysis; trypsin; lysolecithin.

One way of studying interaction between hormone and receptor is by preliminary treatment of the plasma membrane of the target cell with a certain enzyme, followed by the investigation of the effect of the substrate or end product of the enzymic reaction on hormonal stimulation. Treatment of adipose tissue (or of isolated adipocytes) with proteases [7], phospholipases [3, 9], and neuraminidase [4] has revealed the role of protein, lipid, and carbohydrate components of the membrane in the mechanism of reception of the signal and its transmission from the receptor.

In the investigation described below the lipolytic action of adrenalin and ACTH on rat adipose tissue was studied *in vitro* after preliminary treatment with trypsin, which affects the protein components of the adipocyte membrane. The magnitude of the lipolytic response to the action of these hormones also was studied after treatment of the sample with various phospholipids known to influence hormonal stimulation and capable even of imitating the action of certain hormones [6, 8].

METHOD

Rats weighing 170-180 g, deprived of food for 24 h but given water *ad lib*, were killed by decapitation, after which the epididymal adipose tissue was removed, the thin distal part was cut off, and pieces weighing 100 mg were transferred to 2 ml Krebs—Ringer solution, pH 7.4 [2]. After incubation for 15 min with trypsin (Boehringer, West Germany) soybean trypsin inhibitor (Calbiochem, USA) was added to the samples in the ratio of 1:1; after 10 min, pieces of fat were washed in warm Krebs—Ringer solution and transferred to a fresh solution of buffer to which hormone had been added. The samples were incubated with shaking for a further hour, then placed in ice, and the content of nonesterified fatty acids (NEFA) in 100-mg samples of adipose tissue was determined by Duncombe's method [5]. The concentration of L-adrenalin (Calbiochem, USA) in the sample was 0.1-1.0 μg/ml, and that of ACTH (93 i.u./mg, from Serva, West Germany) was 0.1-0.4 μg/ml. Phospholipids were added to the incubation medium after sonication on the Sonic-300 Dismembrator (USA; 22 kHz, 220 μA) in a concentration of 0.06-0.6 mM.

RESULTS

Graphs showing dependence of the lipolytic action of the hormones on their concentration in the presence of trypsin (1 mg/ml) and on the trypsin concentration in concentrations of hormone stimulating lipolysis minimally are illustrated in Fig. 1. Treatment of the tissue with trypsin was shown to reduce the lipolytic action of ACTH by 95% in the presence of trypsin in a concentration as low as 0.1 mg/ml, whereas the lipolytic action of adrenalin was reduced by 50% in the presence of 0.5 mg/ml trypsin, and was unchanged with an increase in its

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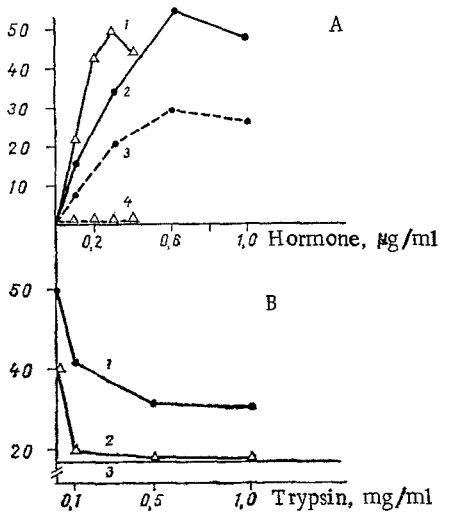

Fig. 1. Effect of trypsin on hormone-stimulated lipolysis in rat epididymal adipose tissue. A: 1) ACTH; 2) adrenalin; 3) adrenalin + trypsin (1 mg/ml); 4) ACTH + trypsin (1 mg/ml). B: 1) Adrenalin (1 μg/ml); 2) ACTH (0.4 μg/ml); 3) basal lipolysis. Ordinate, μmoles NEFA/100 mg wet weight of tissue/h.

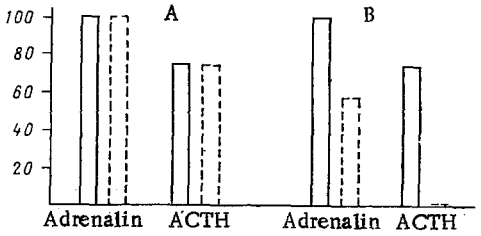

Fig. 2. Effect of suspension of phospholipids on hormone-stimulated lipolysis in rat epididymal adipose tissue. Solid columns) control, broken columns) addition of phospholipid. A) Phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, and lysophosphatidylethanolamine; B) lysolecithin. Ordinate, effect of hormone (%).

concentration to 2 mg/ml. This is evidence of differences in the structure of the adrenoreceptors and the ACTH receptor sites on the plasma membrane of the adipocytes. It can tentatively be suggested, in particular, that the active fragment in the ACTH receptor site contains lysine and arginine, the peptide bond between which is broken by trypsin. It is interesting to note that these basic amino acids are regarded as essential components for postreceptor responses in the phospholipid—Ca^{++} system [1], whereas transmission of the signal for ACTH in the membrane from the receptor to adenylate cyclase is dependent on the presence of Ca^{++}. The results of investigation of the effect of a suspension of phospholipids on hormone-stimulated lipolysis in rat epididymal adipose tissue are given in Fig. 2.

The addition of a suspension of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and lysophosphatidylethanolamine in a concentration of 0.06-0.6 mM did not affect the velocity of the lipolytic reactions whether in the presence or the absence of hormones. However, the addition of a suspension of lysolecithin led to complete loss of sensitivity of the tissue to ACTH and to a decrease in sensitivity to adrenalin on average

by 40%. Lysolecithin is known to have a membranolytic action, but the exact mechanism of this action is unknown. Differences in the degree of change of sensitivity of adipose tissue to ACTH and adrenalin after treatment with lysolecithin are thus evidence merely that the organization of the membrane part of the mechanism responsible for the action of adrenalin is less sensitive to disturbance of its structure by lysolecithin than in the case of ACTH.

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CHARACTERISTICS OF HUMAN SPLENIC PROTEINASES ACTIVE IN A NEUTRAL MEDIUM*

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Fractionation of proteins of aqueous and salt (1 M KCl) extracts of human spleen on Sephadex G-100 revealed several proteinases hydrolyzing histone and casein and active in a neutral medium. The enzymes were found in the extracts in a relatively inactive form, due to the presence of an inhibitor, chiefly in the aqueous extract. Proteinases active in a neutral medium were found in two protein fractions. "High-molecular-weight" proteinases are inhibited by di-isopropylfluorophosphate (DFP), so that they can be classed in the group of serine proteinases. The fraction of "low-molecular-weight" proteinases contains neutral SH-dependent proteinase (proteinases) and enzymes inhibited by DFP. Kininogenase activity and activity hydrolyzing N-Boc-L-alanine nitrophenyl ester, N-benzoyl-L-tyrosine ethyl ester, and N-benzoyl-DL-arginine-p-nitroanilide also were found in this fraction.

KEY WORDS: neutral proteinases; human spleen.

Studies of proteolytic enzymes of bovine spleen in the protein fraction with a molecular weight of about 25,000 have revealed a neutral SH-dependent proteinase hydrolyzing histones [1] and a proteinase with kininogenase activity [2].

Considering the possible role of these and certain other enzymes in the development of various pathological states, it was decided to study whether these proteinases are present in the human spleen and also to attempt to determine the general characteristics of proteinases active in a neutral medium.

*The following abbreviations are used: NBA) N-Boc-L-alanine nitrophenyl ester; BTEE) N-benzoyl-L-tyrosine ethyl ester; BAPA) N-benzoyl-DL-arginine-p-nitroanilide; DFP) di-isopropylfluorophosphate; p-CHMB) p-chloromercuribenzoate; DTT) dithiothreitol.

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