Importance of the cyclic AMP concentration for the rate of lipolysis in human adipose tissue

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Summary

1. The activation of lipolysis on incubation of human subcutaneous adipose tissue was examined in terms of the relationship between the release of glycerol and the concentration of tissue cyclic AMP.

2. A strong positive correlation was obtained between the maximum concentration of cyclic AMP and the rate of glycerol release in the presence of noradrenaline \( r = 0.9 \), whereas, in the basal state, these two parameters were only weakly correlated \( r = 0.45 \).

3. It appears that the noradrenaline-induced rate of lipolysis depends upon the maximal concentration of cyclic AMP that is present in human adipose tissue.

Key words: adipose tissue, cyclic AMP, lipolysis.

Introduction

It is generally recognized that the activation of lipolysis in adipocytes is mediated by an intracellular accumulation of cyclic AMP (Robison, Butcher & Sutherland, 1971). However, the concentration of cyclic AMP and activation of lipolysis are not well correlated in rat adipocytes (Fain, 1973) and it has even been proposed that the concentration of cyclic AMP is of minor importance for the control of the rate of lipolysis (Fredholm, 1978). In the present study the relationship between the rate of lipolysis and the concentration of cyclic AMP has been examined in human adipose tissue incubated in vitro, in the absence and presence of noradrenaline.

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Materials and methods

Subcutaneous adipose tissue was obtained from 20 patients undergoing either cholecystectomy or operations for hernia. The patients were otherwise healthy. There was no selection on the basis of age, sex or body weight. They were fasted overnight. Sodium chloride solution (9 mg/ml: saline) only was given intravenously until tissue specimens (approximately 5 g in weight) were taken at the start of the operation. A short-acting mixture of a barbiturate and phenylanil (Leptanal, LEO, Sweden) was given intravenously as an anaesthetic. The study was approved by the Ethical Committee of the Karolinska Institute. The specimens were conveyed to the laboratory in saline and divided into portions of 50 mg. All incubations were run in quadruplicate with air as the gas phase. The number of adipocytes incubated was calculated from the mean cellular triglyceride content and the total triglyceride content of the tissue portions (Hirsch & Gallian, 1968). Noradrenaline bitartrate was added after pre-incubation in vitro, to give a final concentration of 6 \( \mu \)mol/l. Intracellular water in human subcutaneous adipocytes was calculated to be 70 \( \mu \)l/g of lipid from the data of Englhardt, Liebermeister, Reuter & Irmscher (1971).

For the determination of lipolysis 100 mg of tissue was pre-incubated in Krebs–Henseleit bicarbonate buffer (37°C; pH 7.4) containing 40 mg of dialysed bovine serum albumin/ml for 30 min and then incubated for 2 h in 1 ml of fresh buffer of the same type. Two samples of the medium were removed for the estimation of glycerol (Chernick, 1969). The pH of the medium did not change during incubation.

For the determination of cyclic AMP, 200 mg
of tissue was pre-incubated in Krebs–Henseleit bicarbonate buffer for 30 min and then incubated in 2.5 ml of fresh buffer of the same type for 10 min. The tissue concentration of cyclic AMP was determined in duplicate by a protein-binding method (Arner & Östman, 1975).

Glucose was omitted from the buffers since this was known to influence the rate of lipolysis but not the accumulation of cyclic AMP in intact human adipose tissue (Arner & Östman, 1976). We have demonstrated before: (1) that catecholamine-induced cyclic AMP concentrations in segments of human adipose tissue reach a peak after 10 min of incubation and (2) that basal and catecholamine-stimulated rates of lipolysis are linear for at least 4 h of incubation in the absence of glucose (Arner, 1976).

Linear regression analyses were performed by the method of least squares. Values are expressed as means ± se.

Results

In adipose tissue (Fig. 1) that was incubated in the presence of noradrenaline there was a positive correlation between the log concentration of tissue cyclic AMP at 10 min and release of glycerol at 2 h ($r = 0.90, P < 0.001$). A similar but weaker correlation ($r = 0.45, P < 0.05$) was observed for incubation under basal conditions. There was also a positive correlation ($r = 0.89, P < 0.001$) between the log concentration of tissue cyclic AMP and the rate of glycerol release, in respect of the net effect of noradrenaline. The slopes of the lines in Fig. 1 (a) and (b) were determined for the most part by including the two extreme right points. However, even if these points were excluded the values exceeded the significance limit for $P < 0.05$ ($r = 0.68$, in Fig. 1b and $r = 0.63$, in Fig. 1c). The average concentration of cyclic AMP in the intracellular water space in the experiments in Fig. 1 was calculated to be $1.1 ± 0.1 \mu$mol/l in the basal state and $1.9 ± 0.4$ in the presence of noradrenaline, assuming that cyclic AMP was equally distributed in the intracellular water.

Discussion

The intracellular regulation of the rate of hormone-induced lipolysis has been the subject of considerable research in the past. It has been proposed that possible modulators of the rate of lipolysis are prostaglandins, free fatty acids and adenosine (Fain, 1973; Fredholm, 1978). Cyclic AMP has been ascribed to be of little importance as a regulator, since a quantitative link between the concentration of cyclic AMP and lipolysis exists only over a fraction of the range (Fain, 1973; Fredholm, 1978).

It has been suggested (Fain, 1973) that hormone stimulation is followed by an unphysiological overproduction of cyclic AMP. However, these conclusions are based on studies in the rat (Fain, 1973). In the present investigation on human adipose tissue a strong correlation is demonstrated between the maximum noradrenaline-induced accumulation of cyclic AMP and the noradrenaline-induced increase in the rate of lipolysis. The data clearly indicate that the
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extent to which hormone-sensitive lipase is activated in human adipose tissue after catecholamine stimulation is dependent on the magnitude of cyclic AMP accumulation. This does not rule out the possibility of other substances also exerting a regulatory role. The average noradrenaline-induced peak concentration of cyclic AMP in the intracellular water (1.9 μmol/l) is within the range of protein kinase activation since it is demonstrated that half-maximal and maximal activation of protein kinase in human subcutaneous adipose tissue is achieved with 0.3 and 2.5 μmol of cyclic AMP/1 respectively (Khoo, Aguino & Steinberg, 1974). In contrast to the findings with noradrenaline, basal cyclic AMP and basal lipolysis were only weakly correlated. Possible explanations for this are compartmentalization of basal cyclic AMP, influence of stromal cyclic AMP and the existence of cyclic AMP-independent mechanisms for the activation of lipolysis, which has been demonstrated in the rat (Wise & Jungas, 1978). It is also possible that anaesthetics may influence basal lipolysis by a cyclic AMP-independent mechanism.

The semi-logarithmic relationship between cyclic AMP and lipolysis indicates that cyclic AMP stimulates lipase more effectively at low, rather than at high, rates of lipolysis. With human adipose tissue Khoo et al. (1974) have demonstrated a hyperbolic relationship between lipase activation, on the one hand, and protein kinase or cyclic AMP on the other. These findings could partially account for the semi-logarithmic relationship between the concentration of cyclic AMP and lipolysis in man. A non-linear relationship between the accumulation of cyclic AMP and the lipolytic response has been observed before in isolated rat adipocytes (Fredholm, 1978). Several authors have found that stimulation of basal lipolysis in those cells to 50% of the maximum rate corresponds to a 25% increase in the basal concentration of cyclic AMP (Fredholm, 1978). Thus it would appear that the concentration of cyclic AMP is most important in the regulation of lipolysis stimulated at low rates in human and rat adipose tissue.

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References


