Insulin-stimulated high affinity cyclic AMP phosphodiesterase in rat mammary acini

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High affinity cyclic AMP phosphodiesterase activity in preparations of acini isolated from mammary tissue of lactating rats is shown to be stimulated by the addition of physiological concentrations of insulin to incubations of acini in vitro. This effect is expressed specifically on membrane-associated phosphodiesterase and occurs in the absence of concurrent protein synthesis. The possible functional role of this aspect of insulin's action on mammary tissue is discussed and compared with the well-known reversal by this hormone of the effects of lipolytic agents in adipose tissue and liver.

Insulin *Cyclic AMP phosphodiesterase* *Mammary acini*

1. INTRODUCTION

Acute regulation of cellular metabolism in response to hormonal and other stimuli is commonly mediated by changes in the cytosolic concentration of either cyclic AMP or Ca^{2+} with possibilities for interaction between these two second messengers [1,2]. Current hypotheses link the activation of Ca^{2+} or cyclic AMP-dependent protein kinases with the final delivery system of these second messengers [3]. The identity of the second messenger for the action of insulin, however, has not yet been firmly established. There is long-standing evidence of changes in the phosphorylation status of certain target enzymes in adipocytes [4,5] and hepatocytes [6] following exposure to insulin in vitro. More recent data implicate a small molecular mass acid-stable chemical messenger, liberated from the plasma membrane on treatment with insulin, in the mediation between the binding of insulin to its plasma membrane receptors and the phosphorylation/dephosphorylation, with consequent activity change, of target proteins [7–9].

One such target protein is a high affinity form of cyclic AMP phosphodiesterase (EC 3.1.4.17). In adipocytes [10] and hepatocytes [11] the activity of this enzyme in a membrane fraction is enhanced by treatment with insulin (review [29]). In adipocytes, the insulin-sensitive phosphodiesterase occurs in both plasma membrane and endoplasmic reticulum [10,12,29]; also in this cell type, the involvement of the low molecular mass chemical mediator in insulin-dependent activation of high affinity cyclic AMP phosphodiesterase has been reported [13]. All but one report [14] of insulin-sensitive phosphodiesterase in the hepatocyte locate it on the plasma membrane. Authors in [15] claim that its insulin-activation in hepatocyte plasma membranes is accompanied by a cyclic AMP-dependent phosphorylation which is mediated not by soluble cyclic AMP-dependent protein kinase but by a membrane-associated protein kinase which, it is postulated, may be in a close stoichiometric association with the insulin receptor.

It seems likely that several distinct mechanisms

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may be involved in the response of susceptible cells to insulin. In the light of its effect on cyclic AMP phosphodiesterase, it remains a possibility that one such mechanism, particularly associated with the antagonism of the effects of catecholamines and glucagon, is the lowering of cyclic AMP levels in select intracellular compartments.

We describe here an investigation of the response to insulin challenge of the cyclic AMP phosphodiesterase activity in acini isolated from lactating rat mammary gland. These studies were undertaken firstly to extend the generality of the observations reported using adipocytes and hepatocytes and secondly because of the unusual profile of hormone-response in this mammary tissue preparation [17] characterized by sensitivity to insulin but apparently not to adrenaline or glucagon.

2. EXPERIMENTAL

2.1. *Preparations and procedures*

Acini were prepared from the inguinal/abdominal mammary glands of mid-lactating (9–11 days post-delivery) Wistar rats by collagenase digestion as in [18] and [17]. They were incubated in a Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 4% (w/v) dialyzed bovine serum albumin and 2% (w/v) dialyzed Ficoll at 37°C in the presence or absence of additions as indicated. Each incubation vessel (25 ml stoppered polycarbonate conical flask) contained 2 ml medium, an O_2/CO_2 mixture (95%/5%) in the gas phase, and was shaken at 160 excursions per min in a shaking water bath. At time zero, 0.5 ml of a suspension containing about 250 mg wet wt of acini was added to the flask. After 60 min, the entire contents of each flask were homogenized in a glass-glass Potter-Elvehjem-type homogenizer with a motor driven pestle (1000 rpm) at 0°C for 45 s.

The resulting homogenate was either used directly for assays or subjected to centrifugation at $178\,000 \times g$ for 2 min at 4°C in a Beckman Airfuge to sediment a crude particulate fraction, which was then resuspended to the original homogenate volume, in 40 mM Tris–HCl (pH 8.0) containing 4.3 mM 2-mercaptoethanol. In a given experiment, each experimental condition (control, plus insulin, etc.) was represented by duplicate flasks. Each experiment was performed using a different preparation of acini.

2.2. *Assay methods*

Suitable dilutions of unfractionated acini homogenates or of particulate fractions were assayed in duplicate at 30°C for cyclic AMP phosphodiesterase activity at 1 μM cyclic AMP using the two-step procedure in [19]. 5′-Nucleotidase (EC 3.1.3.5) was assayed at 37°C as in [20] and the protein content of particulate fraction suspensions was measured by the dye-binding method in [21]. It was not possible to determine the mammary cell protein content of homogenate samples because of the presence in them of 4% albumin. Phosphodiesterase activities are expressed, therefore, either as μU per ml homogenate or per mU 5′-nucleotidase activity. One unit of enzyme activity is that which generates 1 μmol product/min under the defined conditions of assay.

2.3. *Chemicals and radiochemicals*

Cyclic [8-^{3}H]AMP (spec. act. 28 Ci/mmol) was purchased from Amersham International (Amersham, England). Dowex 1x8-400 (chloride form), cyclic AMP, bovine serum albumin (fraction V, essentially fatty acid-free) cycloheximide and Ficoll type 400 were from Sigma (Poole, England). The Bradford protein reagent was from Bio-Rad (Watford, England) and collagenase was Worthington CLS III (lot no. 41S130). Insulin (porcine, 23.6 U/mg) was a generous gift from the Boots Co. Ltd (Nottingham, England). All other chemicals were of Analar purity from BDH Ltd (Poole, England).

3. RESULTS

When mammary acini were incubated in the presence of insulin, under conditions which have been shown to permit the expression of an insulin-stimulation of lipogenesis [17], high affinity cyclic AMP phosphodiesterase activity was increased relative to that of the insulin-free control. This effect was observed only when phosphodiesterase activity of particulate fractions was assayed; an increase was not detectable in unfractionated homogenates of insulin-treated acini (table 1). No effects of insulin either on unfractionated acini or particulate fractions were seen when phosphodiesterase activity was measured using 100 μM cyclic

Table 1

Effect of insulin and cycloheximide on high affinity cyclic AMP phosphodiesterase activity in rat mammary acini

Additions	No. of experiments	'Low K_m' cyclic AMP phosphodiesterase activity				
		Unfractionated homogenate		Particulate fraction		
		μU/ml	μU/mU 5'-nucleotidase	μU/ml homogenate	mU/mg protein	μU/mU 5'-nucleotidase
None	8	744.3 ± 96.7	2.71 ± 0.52	115.3 ± 14.3	0.24 ± 0.04	1.00 ± 0.12
Insulin	8	750.4 ± 96.4	2.72 ± 0.54	146.0 ± 15.4****	0.30 ± 0.03**	1.37 ± 0.16****
Cycloheximide	4	661.7 ± 44.7	2.72 ± 0.24	130.9 ± 17.4	0.20 ± 0.03	0.77 ± 0.13
Cycloheximide plus insulin	4	825.1 ± 68.8†††	3.43 ± 0.32††††	175.0 ± 19.8†	0.28 ± 0.02 †	1.00 ± 0.1††

Acini were incubated as described in the text, with insulin (1.68 mU/ml) and/or cycloheximide (50 μg/ml) as indicated. Values given are means ± SE. Statistically significant differences were determined using Student's *t*-test for paired observations. Asterisks denote values greater than the control (no addition) sample at $p < 0.005$ (****) or $p < 0.025$ (**). Daggers denote values greater than the 'plus cycloheximide' sample at $p < 0.005$ (††††), $p < 0.01$ (†††), $p < 0.025$ (††) or $p < 0.05$ (†). 5'-Nucleotidase values in all homogenates, averaged 242.9 mU/ml.

AMP (not shown). Homogenates of rat mammary tissue and of isolated acini have been shown [22] to contain two major kinetic forms of cyclic AMP phosphodiesterase, with $K_{0.5}$ values for cyclic AMP of 1.3 μM and 21.6 μM. Thus, activity measured as in this study at 1 μM cyclic AMP (table 1) reflects predominantly the activity of those forms of cyclic AMP phosphodiesterase having a high affinity for cyclic AMP.

Exposure of acini to insulin for a shorter duration (15 min) than that used in the above experiments (60 min) did not in our initial experiments consistently result in a stimulation of cyclic AMP phosphodiesterase (not shown). This was unexpected, since the analogous effect in hepatocytes and adipocytes is reported to be rapid, reaching maximal amplitude in hepatocytes for instance [11] within 2–5 min at 37°C. Insulin is known to be capable of a generalized stimulation of protein synthesis in several cell-types including adipocytes (see, e.g. [23]). It seemed possible that the apparently 'slow' effect of insulin on mammary cell phosphodiesterase was an expression of this aspect of insulin's action. That this was not so was demonstrated by a series of experiments in which cycloheximide was included in the incubations of mammary acini with and without insulin. The protein synthesis inhibitor failed to suppress the stimulation by insulin of cyclic AMP phosphodiesterase activity; indeed, it significantly enhanced this activity (table 1). Qualitative aspects of these results were essentially unchanged whether enzyme activity data were expressed on a 'per incubation', a 'per mg protein' or a 'per unit of 5'-nucleotidase activity' basis.

4. DISCUSSION

The physiological role of the stimulation of cyclic AMP phosphodiesterase by insulin remains the subject of debate. Undoubtedly, such stimulation does provide a possible mechanism for the insulin-mediated reversal of the actions of those hormones (e.g. glucagon, adrenaline) which act primarily to increase the intracellular concentration of cyclic AMP, in that the physiological effects of insulin-treatment in both adipocytes and hepatocytes are accompanied by a decrease in intracellular cyclic AMP [24,25]. An analogous effect may exist in mammary acini: although glucagon and adrenaline are apparently without effect on this preparation [17,26,27], theophylline has been shown to diminish the rate of lipogenesis by acini in vitro [17]. Since this agent is an inhibitor of cyclic nucleotide phosphodiesterases [28], its effect on lipogenesis most probably results from an increase in the intracellular concentration of cyclic AMP. Insulin is able to reverse this theophylline-induced inhibition of lipogenesis in mammary acini [17]. Thus, this report of the oc-

currence of an insulin-stimulated cyclic AMP phosphodiesterase in a tissue (mammary gland) in which the effects of a lipolytic agent have been shown to be reversed by insulin strengthens the hypothesis that the two phenomena may be mechanistically linked.

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