THE EFFECT OF AN INSULIN-SENSITIVE CHEMICAL MEDIATOR FROM RAT ADIPOCYTES ON LOW K_m AND HIGH K_m CYCLIC AMP PHOSPHODIESTERASE

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1. Introduction

The addition of physiological concentrations of insulin to an adipocyte subcellular system consisting of plasma membranes and mitochondria has been shown to rapidly dephosphorylate the α-subunit of the mitochondrial enzyme pyruvate dehydrogenase [1,2] and to increase enzyme activity [3,4]. This effect could be demonstrated only when plasma membranes were present [2] and could be mimicked by other insulin-like ligands [3]. These data suggested that an insulin-sensitive material was generated from the plasma membranes and that this material may represent the putative chemical mediator of insulin action.

Subsequent work has shown that insulin treatment of intact adipocytes [5] and adipocyte plasma membranes [6,7] increased the amount or activity of a low M_r (1000–1500), acid-stable material which stimulated pyruvate dehydrogenase in mitochondria from adipocytes. To substantiate that this material represents the chemical mediator of insulin action, two criteria must be met:

(i) The quantity of the material recovered from cells should differ according to the insulin sensitivity of these cells. Such differences have been found following extraction of the mediator from insulin-treated and control cells. The insulin-insensitive cells used were IM9 lymphocytes [8] and the insulin-sensitive cells studied include skeletal muscle in [9,10] and adipocytes and hepatoma cells in [5,11].

(ii) The mediator should modulate insulin-sensitive enzymes in a manner analogous to that induced by insulin treatment of whole cells. The mediator alters the activity of 3 insulin-sensitive enzymes, glycogen synthase [9], pyruvate dehydrogenase [3–7,10] and [Ca^{2+} + Mg^{2+}]ATPase [12].

This study expands the number of insulin-sensitive enzymes tested by measuring the effects of the mediator from adipocytes on the low K_m and high K_m cyclic AMP phosphodiesterase.

2. Materials and methods

Male Sprague-Dawley rats (120 g) were obtained from Harlan Industries (Madison WI). Most chemicals including albumin (fraction V) and collagenase were purchased from Sigma; porcine insulin was a gift from Dr R. Chance of Eli Lilly; [1-^{14}C]pyruvic acid and cyclic [2,8-^{3}H]AMP were purchased from New England Nuclear; Norit charcoal and formic acid (90%) were purchased from Fisher; organic scintillator solution was from Amersham. The lots of collagenase and albumin used were chosen as in [3].

Isolated adipocytes and adipocyte plasma membranes, mitochondria and microsomes were prepared as in [13] except that EDTA was omitted. The assay for pyruvate dehydrogenase and the method for extracting the chemical mediator from partially purified plasma membranes [6] and from insulin-treated and untreated (control) adipocytes [5] has been detailed. The low and high K_m cyclic AMP phosphodiesterase were assayed in the microsomal fraction as in [14]. The concentration of cyclic AMP used for the low K_m assay was 0.1 mM and for the high K_m assay, 250 mM; 0.1 μCi and 1.0 μCi of cyclic [^{3}H]AMP was added per assay tube, respectively.

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3. Results

3.1. *The effect of the chemical mediator from intact adipocytes on low* K_m *cyclic AMP phosphodiesterase*

Table 1 demonstrates the effect of 4 Sephadex G-25 fractions of extracts from insulin-treated and control adipocytes on low K_m cyclic AMP phosphodiesterase. Fraction III from the insulin-treated cells resulted in the greatest activation of low K_m cyclic AMP phosphodiesterase compared to the other fractions. The greatest difference between the insulin-treated and control cells was observed with fraction III. Fraction III corresponds to $M_r \sim 1000$–1500 as indicated by peptide and nucleotide markers. The effect of these same 4 fractions on pyruvate dehydrogenase was determined (not shown). Again, only fraction III from the insulin-treated cells caused significantly greater enzyme activity compared to the same fraction from control cells.

Fig.1 summarizes the dose–response relationship for 3 of the Sephadex G-25 fractions from insulin-treated adipocytes on low K_m cyclic AMP phosphodiesterase. Increasing volumes of 2, 5, 10 and 20 μl of the active fraction, fraction III, from insulin-treated cells produced a progressive rise in enzyme activity above basal levels to a plateau of 42 pmol . mg^{-1} . min^{-1} at 10 μl. In each assay 10 μl fraction III were used unless specified otherwise. Varying the volume of the fractions eluted before or after fraction III showed no significant increase in enzyme activity above basal levels.

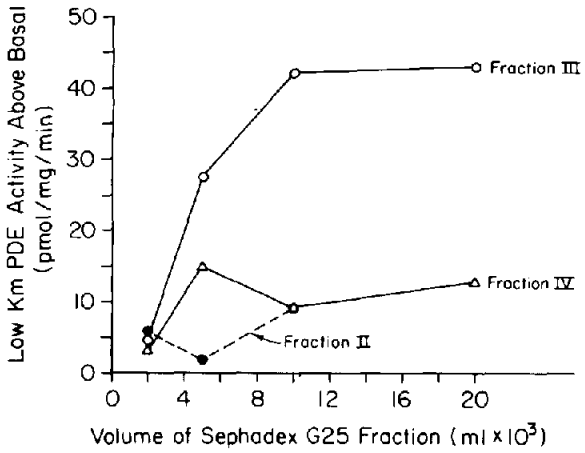

Fig.1. The effect of the quantity of 3 Sephadex G-25 fractions from insulin-treated adipocytes on the activity of the low K_m cyclic AMP phosphodiesterase. The results are from a single representative experiment performed in triplicate. The basal activity was 103.9 ± 1.2 pmol . mg^{-1} . min^{-1}.

3.2. *The effect of the chemical mediator from adipocyte plasma membranes on low* K_m *cylic AMP phosphodiesterase*

Table 2 illustrates the effect of Sephadex G-25 fractions of supernatant derived from adipocyte plasma membranes on pyruvate dehydrogenase and low K_m cyclic AMP phosphodiesterase. Fraction III caused the greatest activation of both pyruvate dehydrogenase and low K_m cyclic AMP phosphodiesterase. Table 3 demonstrates that the active Sephadex G-25 fraction from the supernatant from adipocyte plasma

Table 1
The effect of Sephadex G-25 fractions from insulin-treated and control adipocytes on low K_m cyclic AMP phosphodiesterase

Fraction no.	Insulin-treated	Control (pmol . mg^{-1} . min^{-1})	Change
I	59.6 ± 5.2	54.5 ± 3.9	+5.1
II	52.3 ± 2.9	51.2 ± 8.0	+1.1
III	91.3 ± 5.1	61.7 ± 10.6	+29.6
IV	71.4 ± 2.6	66.7 ± 3.0	+4.7

The results are from a single representative experiment done in triplicate, and the values represent the mean ± SEM. The column labeled 'Change' represents the difference between the insulin-treated group and the control. Of each fraction 10 μl were added. The basal activity was 55.1 ± 4.8 pmol . mg^{-1} . min^{-1} and the activity in the presence of Sephadex G-25 buffer was 59.9 ± 5.9 pmol . mg^{-1} . min^{-1}

Table 2
The effect of Sephadex G-25 fractions of supernatant from adipocyte plasma membranes on pyruvate dehydrogenase and low K_m cyclic AMP phosphodiesterase

Fraction no.	PDH act. (nmol . mg^{-1} . min^{-1})	Low K_m PDE act. (pmol . mg^{-1} . min^{-1})
I	17.4 ± 1.8	95.1 ± 1.6
II	6.5 ± 1.0	83.8 ± 3.1
III	65.1 ± 1.7	117.0 ± 3.3
IV	32.3 ± 3.5	101.3 ± 2.6

The results are from a single representative experiment performed in triplicate, with the values expressed as mean ± SEM. Of each fraction 50 μl were added to the PDH assay. The activity in the presence of Sephadex G-25 buffer was 10.2 ± 1.5 nmol . mg^{-1} . min^{-1}. Of each fraction 10 μl were added to the low K_m PDE assay. The activity in the presence of Sephadex G-25 buffer was 95.5 ± 4.4 pmol . mg^{-1} . min^{-1}

Table 3
Dose–response of Sephadex G-25 fraction III from the supernatant of adipocyte plasma membranes on low K_m cyclic AMP phosphodiesterase activity

Fraction vol. (μl)	Enzyme activity above basal for fraction III (pmol . mg^{-1} . min^{-1})
2	0
5	+14.8
10	+25.9
20	+21.8

The results are from a single representative experiment performed in triplicate. The basal activity was 127.5 ± 1.6 pmol . mg^{-1} . min^{-1}

membranes, fraction III, possesses a dose–response relationship similar to that observed for the active Sephadex G-25 fraction of extracts from insulin-treated adipocytes. In both the experiments using whole cell extract or plasma membrane released mediator, the relative potency of the mediator was equivalent in the pyruvate dehydrogenase and the low K_m cyclic AMP phosphodiesterase assays based on the volumes of the active fractions required for maximal response that were added per unit volume of assay.

3.3. *The effect of the chemical mediator from adipocytes and adipocyte plasma membrane on high* K_m *cyclic AMP phosphodiesterase*

Fraction III from insulin-treated adipocytes failed to significantly increase or decrease the activity of high K_m cyclic AMP phosphodiesterase compared to control (764 ± 17.3 pmol . mg^{-1} . min^{-1} *vs* 782 ± 20.1 pmol . mg^{-1} . min^{-1}, respectively). A similar set of studies has been performed with the material obtained from adipocyte plasma membranes with results identical to those obtained when the extracts from intact adipocytes were assayed.

4. Discussion

This study has shown that a chemical mediator from insulin-treated adipocytes and adipocyte plasma membranes increased the activity of the low K_m cyclic AMP phosphodiesterase in a dose-dependent manner, but had no effect on the high K_m cyclic AMP phosphodiesterase. These changes in enzyme activity parallel the known effect of insulin on the low K_m cyclic AMP phosphodiesterase assayed from intact adipocytes. This enzyme can be added to the list of insulin-sensitive enzymes whose activity is modulated by the chemical mediator further substantiating that this mediator functions as a second messenger for insulin action.

The low K_m cyclic AMP phosphodiesterase from liver plasma membranes is unique in that insulin, as well as epinephrine and glucagon, stimulate its activity [14]. In addition the effect of glucagon and insulin are additive, suggesting separate mechanisms of activation. In [15] insulin was reported to increase the phosphorylation of the enzyme from liver. If insulin activates the low K_m cyclic AMP phosphodiesterase by increasing phosphorylation of the enzyme, then this mechanism would be opposite to that observed for other enzymes that are activated by insulin through dephosphorylation, such as glycogen synthase [9] and pyruvate dehydrogenase [3–7,10]. We are exploring the possibility that phosphorylation of the low K_m cyclic AMP phosphodiesterase from adipocytes alters the activity of the enzyme. If the alteration of low K_m phosphodiesterase by the chemical mediator is by covalent modification, then activation must involve dephosphorylation since no ATP was added in the assay.

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