Inositol trisphosphate activates pyruvate dehydrogenase in isolated fat cells

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Inositol trisphosphate, when added to permeabilized rat fat cells, led to a several-fold increase of pyruvate dehydrogenase activity due to conversion of the inactive phospho form (PDHb) to the active, dephospho form (PDHa). It is suggested that inositol trisphosphate, probably through intracellular Ca^{2+}-mobilisation, acts as a physiological mediator of insulin for activation of the mitochondrial PDH-complex.

Insulin mediator *PDH activation* *Inositol phosphate* *Ca^{2+}-mobilisation* *Isolated fat cell*

1. INTRODUCTION

Receptor-mediated enzymatic hydrolysis of phosphoinositides plays a fundamental role in signal transduction of many hormones, neurotransmitters or other agonists that use calcium as an intracellular messenger (review [1]). Inositol trisphosphate (Ins P_3), one of the cleavage products of phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5 P_2), has been shown to lead to calcium mobilization from intracellular stores of acinar pancreatic cells [2], hepatocytes [3], and isolated microsomes [4]. That Ins P_3 may in fact represent the second messenger for calcium mobilizing hormones has received strong support from recent studies on the action of vasopressin on liver cells [5]. We have previously reported that insulin activates phospholipase C in fat cells, and that this effect had much in common with the activation of pyruvate dehydrogenase (PDH) by insulin [6]. Here, we show that Ins P_3, when added at micromolar concentrations to permeabilized fat cells, leads to PDH activation comparable to that achieved by insulin. Together with other available evidence our results suggest that Ins P_3 may operate as second messenger for the action of insulin on the mitochondrial PDH system in fat cells.

2. MATERIALS AND METHODS

Bovine insulin, bovine serum albumin (BSA), Dowex-50 (H^+ form) were from Sigma, St. Louis, MO, digitonin from Serva, Heidelberg, collagenase CLS from Interchem, München, AG1-X8 anion exchange resin from BIO-Rad, München and [^{32}P]phosphate from Amersham Buchler, Braunschweig. All other chemicals were from Merck, Darmstadt.

2.1. *Preparation and incubation of isolated rat fat cells*

Fat cells were prepared as in [7]. 0.2 ml packed cells were incubated with gentle shaking at 37°C for 10 min in 0.8 ml Hepes buffer [6]. For cell incubation with Ins P_3, Ca^{2+} was omitted from the buffer and 0.25 mg/ml digitonin was added. After incubation the cells were homogenized and PDH activity (PDHa and total activity) was measured spectrophotometrically as in [6].

Fat cell mitochondria were prepared according to [7].

2.2. *Preparation of inositol trisphosphate*

Ins P_3 was prepared from human red cell ghosts according to [8]. Tracer amounts of [^{32}P]Ins P_3

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were obtained after prelabelling of red cells with [^{32}P]phosphate. Ins P_3 was separated by anion exchange chromatography on AG1-X8 (formate form) and freed of ammonium ions with Dowex-50 (H^+-form). The elution pattern of the inositol phosphates corresponded well with that described [9]. Ins P_3 was quantitated on the basis of 3 moles of acid-soluble phosphorus after $HClO_4$-digestion [10] being equivalent to 1 mol of Ins P_3.

3. RESULTS AND DISCUSSION

The effect of Ins P_3 on PDH activity of isolated rat fat cells is shown in fig.1. Accordingly, Ins P_3 caused a more than 3-fold increase of the active form of the enzyme (PDHa) while total PDH activity remained essentially unchanged (274 ± 4.2 mU/g fresh cells, $n = 46$). For comparison, the effect of insulin (only demonstrable on digitonin-free incubations) is shown in panel B of fig.1. It is clear that the conditions used for permeabilization of the cells (calcium starvation and digitonin) did not affect the basal PDHa-levels. Fig.1 further shows that the stimulatory effect of Ins P_3 is abolished in the presence of EGTA. As there was no added calcium available it would appear that Ins P_3, as in other cells [2,3], was mobilizing calcium from intracellular stores and that this was trapped by EGTA. In contrast to [11] these results seem much in favour of calcium being involved in the activation of PDH by insulin.

Another finding shown in fig.1 is the fact that PDH-activation by Ins P_3 did not depend on the presence of glucose, quite different from the effect of insulin which is completely abolished when glucose is omitted from the incubations ([7,12] and fig.2). This may then be explained on assuming that glucose is required as a precursor of inositol phosphate to maintain the phosphoinositide pool at a level sufficient for the release of Ins P_3. This view is consistent with the finding that myo-inositol could replace glucose in permitting PDH-activation by insulin (fig.2). The possibility that myo-inositol may be converted to glucose was excluded by experiments with [U-^{14}C]myo-inositol which yielded no $^{14}CO_2$ on incubation with fat cells (not shown). The concentration dependency of PDH activation by Ins P_3 is shown in fig.3. Accordingly, half-maximal effects were obtained at Ins P_3 concentrations between 1 and 2 μmol/l. Lower

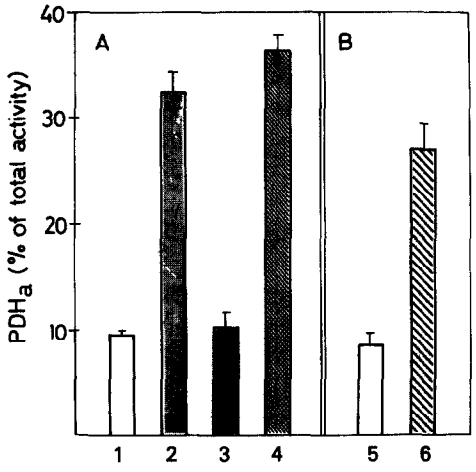

Fig.1. Effects of Ins P_3 on PDH-activities of isolated rat fat cells. (A) Fat cells were incubated in Hepes medium nominally free of calcium containing digitonin, or (B) in regular Hepes medium, as described in section 2. Other experimental conditions were (A) (1) no addition; (2) Ins P_3, 5 μmol/l; (3) Ins P_3, 5 μmol/l plus EGTA, 0.1 mmol/l, (4) Ins P_3, 5 μmol/l, no glucose. (B) (5) no addition, (6) insulin, 100 μU/ml. Mean values ± SE of (A) 4 and (B) 5 experiments are given.

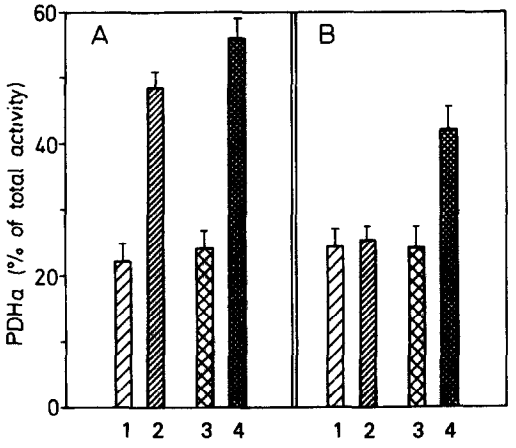

Fig.2. Effect of insulin (100 μU/ml) on PDH activities of isolated fat cells in the presence (A) and absence (B) of glucose, 1 mmol/l. Fat cells were incubated 15 min in Hepes buffer and further treated for PDH determination as described in section 2. Other experimental conditions in A and B, respectively: (1) no insulin, (2) insulin, (3) myo-inositol, 10 mmol/l, (4) myo-inositol, 10 mmol/l plus insulin. Mean values ± SE of 7 experiments are given.

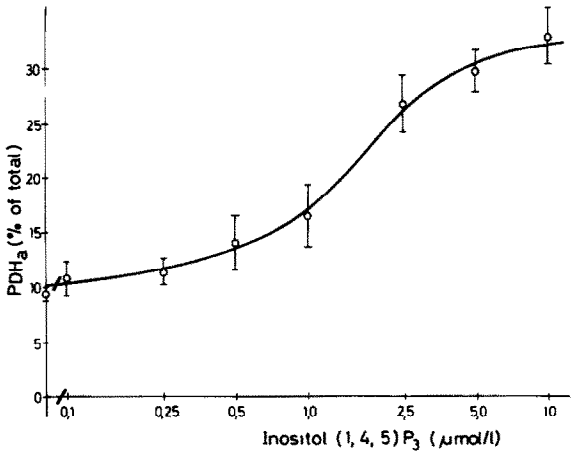

Fig.3. Dose-response relationship of PDH activation by inositol trisphosphate. Isolated fat cells were incubated 10 min in the nominally calcium-free Hepes medium containing digitonin, 0.025%, and further treated as in section 2. Ins P_3 was added at the concentrations indicated on the abscissa. By courtesy of Dr G. Wirthenson we could study a sample of an Ins P_3 preparation that was kindly given to her by Dr Irvine, Cambridge. The results were essentially the same as shown in the figure.

than micromolar concentrations were required for calcium mobilisation [2,3] yet in these studies different kinds of cells and also other permeabilization conditions were used.

In further experiments we have studied if Ins P_3 might also act directly on isolated mitochondria. There was no effect on PDH-activity on incubation of fat cell mitochondria with Ins P_3 at concentrations up to 10 μmol/l neither in the presence nor absence of added calcium (not shown). In contrast, as will be reported elsewhere, 1,2-diolein in the micromolar range did elicit PDH activation when directly added to fat cell mitochondria.

To sum up, it appears very likely that in fat cells, Ins P_3 fulfills the role of a second messenger that transmits the insulin signal to the mitochondrial PDH complex. Although not yet directly shown our data suggest that this effect of Ins P_3, similar to that found in other cells [2,3], involves intracellular calcium mobilization. It should be noted here that calcium mobilization has already been implicated earlier in insulin action yet has still remained a matter of dispute (review, [13]).

Much in support of a second messenger role of Ins P_3 (and perhaps diacylglycerol) in insulin's action appear other studies from this laboratory which have shown that (i) insulin activates phospholipase C in fat cells [6], and (ii) that the insulin receptor associated protein kinase displays the activity of an insulin-stimulatable phosphatidylinositol kinase [14].

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