

# 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  $\text{Ca}^{2+}$ -mobilisation, acts as a physiological mediator of insulin for activation of the mitochondrial PDH-complex.

*Insulin mediator    PDH activation    Inositol phosphate     $\text{Ca}^{2+}$ -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 ( $\text{Ins P}_3$ ), one of the cleavage products of phosphatidylinositol 4,5-bisphosphate ( $\text{PtdIns 4,5 P}_2$ ), has been shown to lead to calcium mobilization from intracellular stores of acinar pancreatic cells [2], hepatocytes [3], and isolated microsomes [4]. That  $\text{Ins P}_3$  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  $\text{Ins P}_3$ , 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  $\text{Ins P}_3$  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 ( $\text{H}^+$  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 [ $^{32}\text{P}$ ]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  $37^\circ\text{C}$  for 10 min in 0.8 ml Hepes buffer [6]. For cell incubation with  $\text{Ins P}_3$ ,  $\text{Ca}^{2+}$  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

$\text{Ins P}_3$  was prepared from human red cell ghosts according to [8]. Tracer amounts of [ $^{32}\text{P}$ ] $\text{Ins P}_3$

were obtained after prelabelling of red cells with [ $^{32}\text{P}$ ]phosphate. Ins  $\text{P}_3$  was separated by anion exchange chromatography on AG1-X8 (formate form) and freed of ammonium ions with Dowex-50 ( $\text{H}^+$ -form). The elution pattern of the inositol phosphates corresponded well with that described [9]. Ins  $\text{P}_3$  was quantitated on the basis of 3 moles of acid-soluble phosphorus after  $\text{HClO}_4$ -digestion [10] being equivalent to 1 mol of Ins  $\text{P}_3$ .

### 3. RESULTS AND DISCUSSION

The effect of Ins  $\text{P}_3$  on PDH activity of isolated rat fat cells is shown in fig.1. Accordingly, Ins  $\text{P}_3$  caused a more than 3-fold increase of the active form of the enzyme (PDH<sub>a</sub>) while total PDH activity remained essentially unchanged ( $274 \pm 4.2$  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 PDH<sub>a</sub>-levels. Fig.1 further shows that the stimulatory effect of Ins  $\text{P}_3$  is abolished in the presence of EGTA. As there was

no added calcium available it would appear that Ins  $\text{P}_3$ , 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  $\text{P}_3$  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  $\text{P}_3$ . 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 [ $\text{U-}^{14}\text{C}$ ]myo-inositol which yielded no  $^{14}\text{CO}_2$  on incubation with fat cells (not shown). The concentration dependency of PDH activation by Ins  $\text{P}_3$  is shown in fig.3. Accordingly, half-maximal effects were obtained at Ins  $\text{P}_3$  concentrations between 1 and 2  $\mu\text{mol/l}$ . Lower

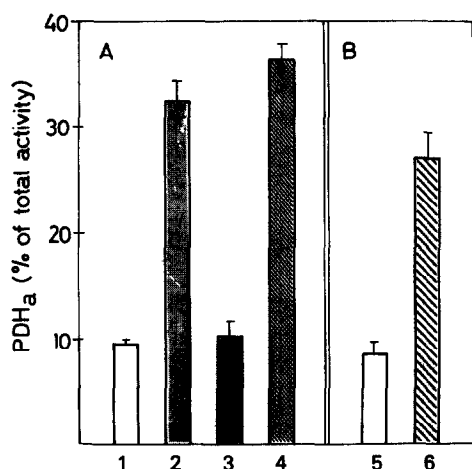


Fig.1. Effects of Ins  $\text{P}_3$  on PDH-activities of isolated rat fat cells. (A) Fat cells were incubated in HEPES medium nominally free of calcium containing digitonin, or (B) in regular HEPES medium, as described in section 2. Other experimental conditions were (A) (1) no addition; (2) Ins  $\text{P}_3$ , 5  $\mu\text{mol/l}$ ; (3) Ins  $\text{P}_3$ , 5  $\mu\text{mol/l}$  plus EGTA, 0.1 mmol/l, (4) Ins  $\text{P}_3$ , 5  $\mu\text{mol/l}$ , no glucose. (B) (5) no addition, (6) insulin, 100  $\mu\text{U/ml}$ . Mean values  $\pm$  SE of (A) 4 and (B) 5 experiments are given.

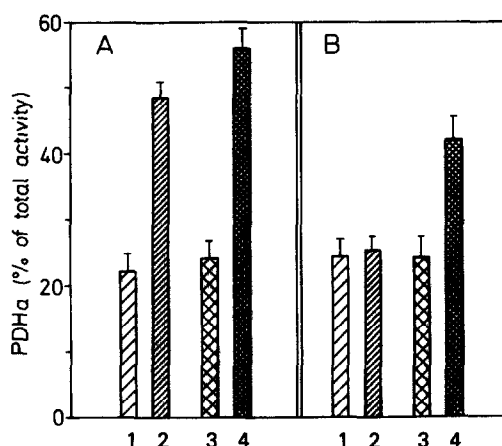


Fig.2. Effect of insulin (100  $\mu\text{U/ml}$ ) on PDH activities of isolated fat cells in the presence (A) and absence (B) of glucose, 1 mmol/l. Fat cells were incubated 15 min in HEPES buffer and further treated for PDH determination as described in section 2. Other experimental conditions in A and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

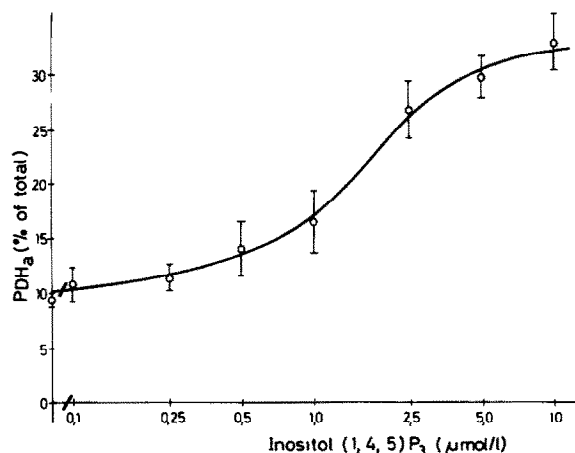


Fig.3. Dose-response relationship of PDH activation by inositol trisphosphate. Isolated fat cells were incubated 10 min in the nominally calcium-free Hepes medium containing digitonin, 0.025%, and further treated as in section 2. Ins P<sub>3</sub> was added at the concentrations indicated on the abscissa. By courtesy of Dr G. Wirthenson we could study a sample of an Ins P<sub>3</sub> 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<sub>3</sub> might also act directly on isolated mitochondria. There was no effect on PDH-activity on incubation of fat cell mitochondria with Ins P<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>, 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<sub>3</sub> (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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