Effect of adenosine on insulin activation of rat adipocyte pyruvate dehydrogenase

Ellen H.-A. Wong*, Judith A. Smith and Leonard Jarett

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA and **Department of Biochemistry, Medical Faculty, National University of Singapore, Kent Ridge, Singapore 0511, Republic of Singapore*

Received 15 June 1984; revised version received 15 July 1984

Adenosine and its analogue N^6-phenylisopropyladenosine stimulated pyruvate dehydrogenase activity of isolated rat adipocytes. Maximal stimulation was obtained with concentrations between 50 and 100 μ M, with the effect decreasing at higher concentrations. The effects of insulin on this enzyme was modified by adenosine. The concentration of insulin (10 μunits/ml) that produced almost half-maximal stimulation, had little or no effect, when adenosine deaminase was present. Adenosine also enhanced the effect of suboptimal but not optimal concentrations of insulin. Thus, the mechanism of adenosine action on adipocyte pyruvate dehydrogenase could in some way be similar or related to that of insulin.

Pyruvate *Dehydrogenase* *Adenosine* *N^6-Phenylisopropyladenosine* *Adenosine deaminase* *Insulin sensitivity* *Adipocyte*

1. INTRODUCTION

Adenosine is continuously produced by adipose tissue [1]. Various studies have shown that its effects on fat cell metabolism are similar to that of insulin in that it stimulates cyclic AMP phosphodiesterase [2], antagonizes cyclic AMP accumulation and inhibits lipolysis [1,3–10]. In addition, it is also known to change insulin sensitivity in adipose tissue. The antilipolytic activity of insulin is abolished when rat adipocytes were exposed to adenosine deaminase [7,8], whilst the effects of insulin on glucose transport and oxidation are enhanced [3,8,11]. The present study investigates whether adenosine could modify the effects of insulin on pyruvate dehydrogenase activity of isolated rat adipocytes by first determining its mode of action on the enzyme.

2. MATERIALS AND METHODS

Insulin was obtained from Dr. R. Chance, Eli Lilly Co. (Indianapolis, IN) and [1-^{14}C]pyruvic acid from New England Nuclear (Boston, MA). All other chemicals and biochemicals were from standard sources.

Isolated fat cells were prepared as in [12] from the epididymal adipose tissues of fed male Sprague–Dawley rats (150–170 g). Krebs–Ringer bicarbonate buffer pH 7.4 containing 2.5 mM calcium and 4% bovine serum albumin was used. Cells were prepared in the presence of adenosine deaminase (2 units/ml) to minimize the accumulation of endogenous adenosine and its effects. Unless otherwise noted, the fat cells (1.8×10^5 cells/ml) were distributed into separate polyethylene vials, gassed with 5% CO_2 and 95% O_2, and the vials tightly closed with rubber stoppers. Experiments were usually carried out at 37°C for 15 or 30 min, after which the cells were separated by centrifuging in a microfuge and extracted with a solution of 0.2% (v/v) Triton-X100, 2 mM DTT, 2 mM EDTA and 2 mM EGTA in 50 mM $KHPO_4$, pH 7.4. Pyruvate dehydrogenase assay was carried out on the infranatant of the extract as in [13] with minor modifications. Due to the variability with which each batch of cells respond to insulin or adenosine, representative experiments are shown. Experi-

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

ments were repeated at least 3 times with different batches of cells to confirm each observation; other than fig.6 standard errors were not shown since they were <1%.

3. RESULTS AND DISCUSSION

3.1. Time course effect of adenosine on pyruvate dehydrogenase

To study the effects of adenosine on insulin sensitivity of pyruvate dehydrogenase, the time course effect of adenosine was determined in order to select a suitable period for the incubation of fat cells. The adenosine analogue N^6-phenylisopropyladenosine (PIA), not metabolized by adenosine deaminase, was used in these experiments in the presence of adenosine deaminase. This was to reduce the accumulation of endogenous adenosine which could obscure the effects of exogenous adenosine. Fig.1 shows that the effects of adenosine was time dependent. Stimulation of pyruvate dehydrogenase activity by the adenosine analogue was evident within 5 min and was almost two-fold at 60 min after the addition of the analogue. This pattern is similar to that shown by a submaximal dose of insulin (20 μunits/ml) on pyruvate dehydrogenase activity [14].

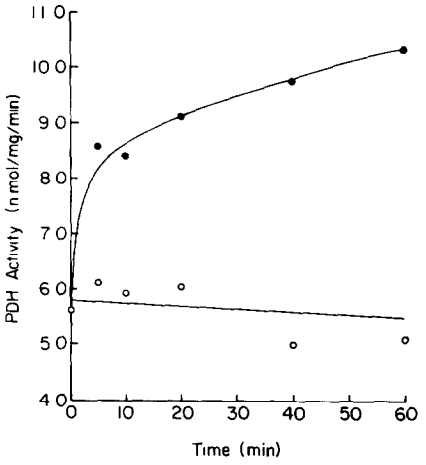

Fig.1. Time course effect of adenosine on isolated adipocyte pyruvate dehydrogenase. Adipocytes (1.8×10^5) were incubated in a final volume of 1.2 ml with PIA (100 μM) and adenosine deaminase (1 unit/ml) for varying time intervals at 37°C. One ml of mixture was extracted with 0.5 ml of stopping agent and assayed for pyruvate dehydrogenase activity. Each point represents the mean of duplicate incubations measured in triplicate. ○, Basal; ●, 100 μM PIA.

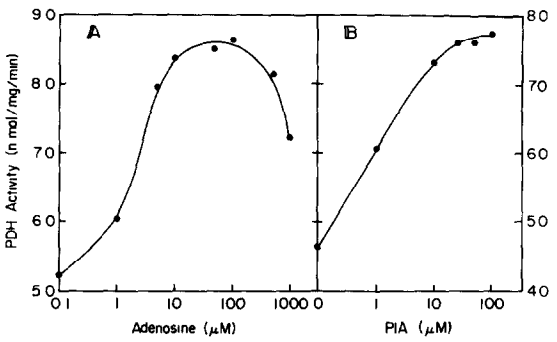

Fig.2. Effect of adenosine concentration on isolated adipocyte pyruvate dehydrogenase. Adipocytes (0.9×10^5) were incubated in the presence of varying concentrations of adenosine (A) or PIA with adenosine deaminase (1 unit/ml) (B) for 15 min. The incubations were stopped, and the cells extracted and assayed for pyruvate dehydrogenase activity as in fig.1. Each point represents the mean of triplicate incubations measured in duplicate.

3.2. Effects of adenosine concentration on the stimulation of pyruvate dehydrogenase

To determine the optimal adenosine concentration for maximal stimulation, the effects of varying amounts of adenosine and its analogue PIA on pyruvate dehydrogenase activity were investigated.

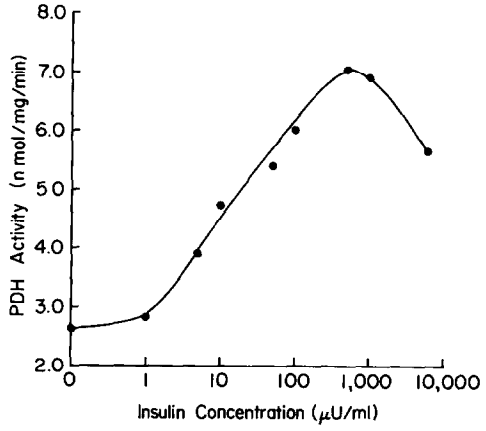

Fig.3. Dose-response to insulin of isolated adipocyte pyruvate dehydrogenase. Adipocytes (1.8×10^5) were incubated for 15 min with varying concentrations of insulin. Values for each point were obtained as in fig.2.

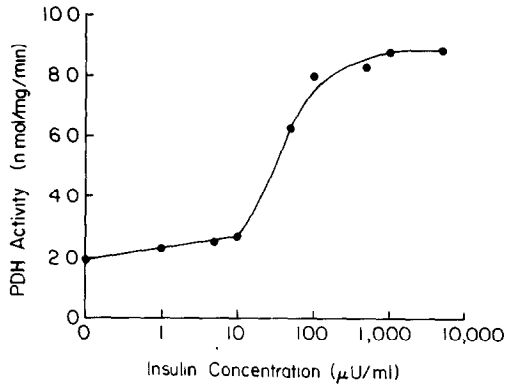

Fig.4. Effect of adenosine deaminase on adipocyte pyruvate dehydrogenase response to insulin concentration. Experimental conditions were similar to those in fig.3 except for the addition of adenosine deaminase (1 unit/ml).

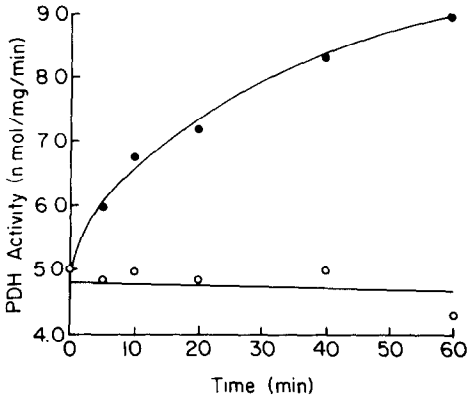

Fig.5. Effect of adenosine deaminase on the time course response of adipocyte pyruvate dehydrogenase to insulin. Experimental conditions were similar to those in fig.1 except that incubations were carried out with insulin (100 μunit/ml) instead of PIA. ○, Basal; ●, 100 μunits/ml insulin.

Results of these experiments are shown in fig.2. The effects of PIA were observed in the presence of adenosine deaminase (fig.2B). Due to its poor solubility, 100 μM was the highest concentration that could be used for the present study. Higher concentrations could, however, be obtained with adenosine (fig.2A). Pyruvate dehydrogenase activation by adenosine was, however, apparent only when the fat cell concentration was 20 mg/ml (10^5 cells/ml) or less and endogenous adenosine accumulation was minimal. In both cases, maximal stimulation was obtained with concentrations between 50 and 100 μM. Higher concentrations of adenosine caused a lesser effect, thereby resulting in a biphasic dose-response (fig.2A).

3.3. The effects of adenosine and adenosine deaminase on the stimulation of pyruvate dehydrogenase activity by insulin

The effect of adenosine on pyruvate dehydrogenase activity, stimulated by various doses of insulin, was investigated by the presence and absence of adenosine deaminase. In the absence of adenosine deaminase, a biphasic insulin dose response was observed (fig.3). When adenosine deaminase was added to the incubating medium, the biphasic effect of increasing concentrations of insulin was not apparent (fig.4). This was probably due to a decrease in insulin sensitivity as indicated by the shift in the dose response curve. In the presence of adenosine deaminase, 10 μunits/ml insulin caused only a small increase in the pyruvate dehydrogenase activity (fig.4), whereas in the absence of adenosine deaminase, a similar concentration of insulin caused a stimulation which was almost half-maximal (fig.3). Thus adenosine appears to increase the sensitivity of the adipocyte to insulin. This phenomenon is also observed when the time course effect of insulin is studied in the presence and absence of adenosine deaminase. The activation of pyruvate dehydrogenase by the optimal insulin concentration of 100 μunits/ml is rapid and

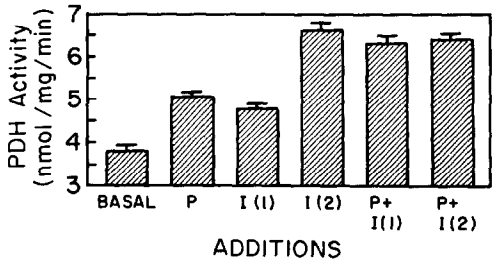

Fig.6. Potentiation of insulin effects on adipocyte pyruvate dehydrogenase by PIA. Experimental conditions were similar to those in fig.2B. Incubations were carried out in the presence of PIA (100 μM), two doses of insulin [suboptimal I(1) 25 μunits/ml and optimal I(2) 100 μunits/ml] and combinations of PIA and insulin. Data shown are the mean + SEM of triplicate incubations measured in duplicate.

reached a peak within 10–20 min [14], whereas the data in fig.5 show that in the presence of adenosine deaminase, the activation of the enzyme was gradual and continued to increase during the 60-min incubation period.

3.4. The inter-relationship of the effect of adenosine with that of insulin on pyruvate dehydrogenase

To determine whether adenosine and insulin could be stimulating pyruvate dehydrogenase activity through a similar or related mechanism, the effects of adenosine were studied in the presence of optimal and suboptimal concentrations of insulin. Fig.6 shows that at the optimal insulin concentration of 100 μunits/ml, adenosine failed to enhance the stimulatory effects of insulin on pyruvate dehydrogenase activity. At the suboptimal insulin concentration of 25 μunits/ml, adenosine caused the enzyme to be maximally stimulated. The failure of adenosine to potentiate the stimulatory effect of insulin at the optimal concentration of 100 μunits/ml, suggests that the mechanism of action by these two agents on pyruvate dehydrogenase could be similar or related. Thus the present study shows that adenosine potentiated the action of insulin by increasing the sensitivity of the adipocyte to insulin and that the mechanism of adenosine action could in some way be similar or related to that of insulin.

ACKNOWLEDGEMENTS

This work was supported by NIH grants AM28144 and AM19525 and a grant from the Reynolds Foundation. E.H.A. Wong is a Visiting Professor from the National University of Singapore and holds an International Fellowship from the American Association of University Women.

REFERENCES

[1] Schwabe, U., Ebert, R. and Erbler, H.C. (1973) Naunyn-Schmiedebergs Arch. Pharmakol. 276, 133–148.
[2] Teo, T.S., Ooi, S.O. and Wong, H.A. (1981) FEBS Lett. 128, 75–78.
[3] Schwabe, U., Schonhoter, P.S. and Ebert, R. (1974) Eur. J. Biochem. 46, 537–545.
[4] Fain, J.N., Pointer, R.H. and Ward, W.F. (1972) J. Biol. Chem. 247, 6866–6872.
[5] Fain, J.N. (1973) Mol. Pharmacol. 9, 595–604.
[6] Ebert, R. and Schwabe, U. (1973) Naunyn-Schmiedebergs Arch. Pharmakol. 278, 247–259.
[7] Schwabe, U., Ebert, R. and Erbler, H.C. (1975) Adv. Cyclic Nucl. Res. 5, 569–584.
[8] Fain, J.N. and Wieser, P.B. (1975) J. Biol. Chem. 250, 1027–1034.
[9] Hjemdahl, P. and Fredholm, B.B. (1976) Acta Physiol. Scand. 96, 170–179.
[10] Turpin, B.P., Duckworth, W.C. and Solomon, S.S. (1976) Clin. Res. 24, 30A.
[11] Joost, H.G. and Steinfelder, H.J. (1982) Mol. Pharmacol. 22, 614–618.
[12] Rodbell, M. (1974) J. Biol. Chem. 239, 375–380.
[13] Kiechle, F.L. and Jarett, L. (1983) Mol. Cell. Biochem. 56, 99–105.
[14] Jarett, L., Wong, E.H.-A., Smith, J.A. and Macauley, S.L., unpublished observations.