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Adenosine and oxytocin reverse antagonism of cyclic AMP elevating agents to insulin activation of adipocyte pyruvate dehydrogenase

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ACTH, isoprenaline, forskolin, and dibutyryl cyclic AMP prevented insulin from stimulating adipocyte pyruvate dehydrogenase in the presence of adenosine deaminase. Antagonism was reversed by N^6-phenylisopropyladenosine as well as oxytocin. The stimulatory effects of insulin, adenosine and oxytocin on adipocyte pyruvate dehydrogenase appear to be through (a) mechanism(s) which is (are) similar or related.

Adenosine; Oxytocin, cyclic AMP; Insulin

1. INTRODUCTION

The ability of glucagon and catecholamines to antagonise the stimulatory effect of insulin on glucose transport appears to be adenosine related in that it is evident only when endogenously produced adenosine is removed with adenosine deaminase [1,2]. On the other hand, the greatest degree of antagonism to isoprenaline inhibition by insulin was observed when insulin was added together with adenosine to cells treated with isoprenaline [3]. The mechanism through which adenosine exerts its effects on glucose transport remains to be determined. It has, however, been suggested that adenosine inhibited adenylate cyclase and consequently reduced cyclic AMP levels and protein kinase activity. This is based on the fact that the inhibitory effect of catecholamines on glucose transport is mimicked by dibutyryl cyclic AMP [4].

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Adenosine has also been shown to activate adipocyte pyruvate dehydrogenase and through (a) mechanism(s) thought to be similar or related to that of insulin [5]. The present study investigated the ability of adenosine to prevent cyclic AMP elevating agents from antagonising the stimulatory effect of insulin on pyruvate dehydrogenase. The effects of oxytocin were also investigated since the hormone has also been shown to activate adipocyte pyruvate dehydrogenase [6,7] but possibly through (a) mechanism(s) separate from that of insulin [8,9].

2. MATERIALS AND METHODS

Insulin was obtained from Dr R. Chance, Eli Lilly Co. (Indianapolis, IN, USA), PIA (N^6-phenylisopropyladenosine) from Boehringer, [1-^{14}C]pyruvic acid from New England Nuclear (Boston, MA, USA), collagenase, adenosine deaminase, hormones and other biochemicals were from Sigma (USA).

Isolated fat cells were prepared by the collagenase method of Rodbell [10] from the epididymal adipose tissues of fed male Sprague-

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Dawley rats (150–170 g). Krebs-Ringer bicarbonate buffer [11], pH 7.4, containing 2.5 mM Ca^{2+} and 4% (w/v) bovine serum albumin was used. Cells were prepared in the presence of adenosine deaminase (2 units/ml) to minimise the accumulation of endogenous adenosine and its effects. Cells so prepared, apart from having a more consistent response to adenosine, did not differ from those prepared in the absence of adenosine deaminase [12].

PIA was used instead of adenosine in the experiments since it has similar effects as adenosine on pyruvate dehydrogenase and is not metabolized by adenosine deaminase [5].

Experimental conditions and assay of pyruvate dehydrogenase activity were as described [13].

3. RESULTS AND DISCUSSION

3.1. *Effects of PIA on antagonism between insulin and cyclic AMP elevating agents on pyruvate dehydrogenase activity*

Adenosine has been reported to reverse the antagonism of catecholamines to insulin-stimulated glucose transport in adipocytes [2,4]. Whether adenosine had similar effects on the regulation of pyruvate dehydrogenase was investigated with PIA in the presence of adenosine deaminase. The effects of PIA on the antagonism of various agents known to elevate cyclic AMP levels are shown in table 1. ACTH, isoprenaline and forskolin completely abolished the stimulatory effect of insulin on pyruvate dehydrogenase, whilst dibutyryl cyclic AMP reduced the effect to slightly above basal levels. In all cases, however, PIA completely reversed the antagonism between these agents and insulin.

From the above results, it would appear that the mechanism through which these agents exerted their effects on pyruvate dehydrogenase activity was cyclic AMP-mediated. The effect of adenosine could then be through the inhibition of adenylate cyclase and consequent reduction in cyclic AMP levels and protein kinase activity. On the other hand, the involvement of Ca^{2+} mobilization has been proposed by Wallace et al. [14] as a result of their study on the inter-relationship between adenosine, glucagon and insulin effects on the plasma membrane cyclic AMP phosphodiesterase in hepatocytes. In the liver, there are no inhibitory R-site receptors coupled to adenylate cyclase activity [15] and desensitization and the blockade of insulin effects by glucagon are therefore not cyclic AMP-mediated [14].

Table 1

PIA reverses antagonism between insulin and cyclic AMP elevating agents

	PDH activity (nmol/min per mg of protein)
Basal	6.56 ± 0.25
Insulin (100 μU/ml)	9.72 ± 0.16
ACTH (2 mU/ml) + insulin	7.12 ± 0.29
ACTH + insulin + PIA[a] (10 μM)	10.01 ± 0.35
Isoprenaline (1 μM) + insulin	7.28 ± 0.40
Isoprenaline + insulin + PIA	9.79 ± 0.34
Forskolin (1 μM) + insulin	6.39 ± 0.17
Forskolin + insulin + PIA	9.91 ± 0.14
DcAMP[b] (1 mM) + insulin	7.91 ± 0.30*
DcAMP + insulin + PIA	9.89 ± 0.35

[a] N^6-phenylisopropyladenosine
[b] Dibutyryl cyclic AMP

Adipocytes were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4, for 10 min in the presence of adenosine deaminase (1 unit/ml) and the agents indicated in the table. Results are means ± SE (n = 6): * $p < 0.02$ vs basal level

3.2. *Effects of oxytocin on antagonism between insulin and cyclic AMP elevating agents on pyruvate dehydrogenase activity*

Oxytocin, like adenosine, has been reported to stimulate pyruvate dehydrogenase in adipocytes [5–7]. It has been suggested that its effect could probably be through (a) mechanism(s) in common with that of insulin [6]. Other studies, however, suggest that insulin-like effects of oxytocin in the fat cell could be through independent systems [8,9] with the possible involvement of calcium [8]. If oxytocin stimulation of pyruvate dehydrogenase is through a system common to that of insulin [6] as is the case with adenosine, there should be a relationship between its mode of action and that of adenosine. Thus oxytocin should also be able to reverse the antagonism between cyclic AMP elevating agents and insulin activation of pyruvate

dehydrogenase. This was indeed found to be the case as shown by the results in table 2. It should be noted, however, that in this series of experiments, the cyclic AMP elevating agents, other than forskolin, did not completely abolish the stimulation by insulin to basal levels of pyruvate dehydrogenase activity. The reason for this could not be established.

3.3. *Effects of PIA on the antagonism between oxytocin and cyclic AMP elevating agents on pyruvate dehydrogenase activity*

The effects of oxytocin on pyruvate dehydrogenase activity in adipocytes in the presence of cyclic AMP elevating agents were investigated since the role of cyclic AMP in oxytocin action is still controversial [9]. Results in table 3 show that oxytocin-stimulated pyruvate dehydrogenase activity was abolished by agents known to elevate cyclic AMP. This antagonism between oxytocin and ACTH or forskolin was completely reversed by PIA. This was, however, not the case with dibutyryl cyclic AMP where a small residual antagonism remained. The difference observed between this agent and the others in their antagonism to oxytocin and insulin effects (tables 1,2) could be due to the difference in its mode of action in elevating cyclic AMP levels in the adipocyte. Dibutyryl cyclic AMP is believed to raise the cyclic AMP level by competitive inhibition of the cyclic AMP phosphodiesterase [16].

3.4. *The inter-relationship of the effects of adenosine and oxytocin with that of insulin on pyruvate dehydrogenase*

The effects of oxytocin and insulin obtained in the presence of maximally effective concentrations of both agents were not additive for either glucose oxidation or lipogenesis [17]. Whether this was also the case for the activation of pyruvate dehydrogenase was investigated.

Results in table 4 show that neither oxytocin or PIA enhanced the effect of insulin at its optimal concentration of 100 μunits/ml. Thus there may be a common mechanism through which adenosine, oxytocin and insulin exert their effects on adipocyte pyruvate dehydrogenase. In the present study, a near maximal concentration of 10 μM adenosine (as determined in [3]) was used for the experiments. This was to avoid the inhibitory effect which sometimes occurred when maximal concentrations of adenosine were used in the presence of optimal concentrations of insulin. The concentration of oxytocin could also have been submax-

Table 2

Oxytocin reverse antagonism between insulin and cyclic AMP elevating agents

	PDH activity (nmol/min per mg of protein)
Basal	3.34 ± 0.02
Insulin (100 μU/ml)	5.26 ± 0.09
ACTH (2 mU/ml) + insulin	4.20 ± 0.25
ACTH + insulin + oxytocin (1 μM)	5.17 ± 0.10*
Isoprenaline (1 μM) + insulin	4.26 ± 0.17
Isoprenaline + insulin + oxytocin	5.23 ± 0.02**
Forskolin (1 μM) + insulin	3.61 ± 0.03
Forskolin + insulin + oxytocin	4.20 ± 0.03**
DcAMP (1 mM) + insulin	4.53 ± 0.02
DcAMP + insulin + oxytocin	5.24 ± 0.06*

Experimental conditions similar to those for table 1. Results are means ± SE ($n = 6$): * $p < 0.01$, ** $p < 0.001$ vs corresponding level without oxytocin

Table 3

PIA reverse antagonism between oxytocin and cyclic AMP elevating agents

	PDH activity (nmol/min per mg of protein)
Basal	5.17 ± 0.28
Oxytocin (1 μM)	7.31 ± 0.32
Oxytocin + PIA (10 μM)	7.88 ± 0.38
ACTH (2 mU/ml) + oxytocin	5.34 ± 0.33
ACTH + oxytocin + PIA	7.72 ± 0.47**
Forskolin (1 μM) + oxytocin	4.86 ± 0.06
Forskolin + oxytocin + PIA	7.63 ± 0.38**
DcAMP (1 mM) + oxytocin	4.52 ± 0.05
DcAMP + oxytocint + PIA	6.65 ± 0.23**

Experimental conditions similar to those for table 1. Results are means ± SE ($n = 6$): ** $p < 0.01$ or better vs corresponding level without PIA, * $p < 0.05$ vs level without DcAMP

Table 4

Inter-relationship between insulin, PIA and oxytocin in the stimulation of adipocyte pyruvate dehydrogenase

	PDH activity (nmol/min per mg of protein)
Basal	5.73 ± 0.43
Insulin (100 μU/ml)	9.12 ± 0.61
PIA (10 μM)	7.54 ± 0.46
Oxytocin (1 μM)	7.32 ± 0.36
Insulin + PIA	9.56 ± 0.64
Insulin + oxytocin	10.04 ± 0.69
PIA + oxytocin	9.35 ± 0.48**
Insulin + PIA + oxytocin	10.23 ± 0.71

Experimental conditions similar to those of table 1. Results are means ± SE ($n = 6$): * $p < 0.05$ vs level with PIA alone, ** $p < 0.02$ vs level with oxytocin alone

imal, as its dose response for maximal effects was found to vary between 0.1 and 10 μM (not shown). This could probably account for the effect of adenosine being enhanced by oxytocin, and vice versa. It should be noted, however, that the effects of these 2 agents added together did not exceed the maximum effects of insulin.

In conclusion, the ability to reverse the antagonism between cyclic AMP elevating agents and insulin effects is not limited to adenosine action alone. The present study shows that oxytocin has similar properties and the mechanism through which these effects are exerted appears to be similar or related. There is no evidence that both adenosine and oxytocin exerted their effects independent of the cyclic AMP system, since antagonism by forskolin and dibutyryl cyclic AMP were effectively reversed.

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