Catecholamines inhibit insulin-stimulated glucose transport in adipocytes, in the presence of adenosine deaminase

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Received 27 December 1982

Epinephrine, norepinephrine and isoproterenol completely inhibited insulin-stimulated 2-deoxyglucose uptake by rat adipocytes, but only when adenosine was prevented from accumulating in the incubation medium by addition of adenosine deaminase. Basal uptake rates were not affected. The effects were not observed in the presence of adenosine deaminase plus N^6-phenylisopropyl adenosine (a non-metabolizable adenosine analogue), suggesting that it is the lowered adenosine level rather than the presence of the enzyme itself which allows the catecholamines to inhibit insulin action. The inhibitory effects of the catecholamines were blocked by propranolol but not phentolamine, suggesting that they are mediated via β-adrenergic receptors.

Insulin *Catecholamine* *Adenosine* *Glucose transport* *2-Deoxyglucose uptake* *Adipocyte*

1. INTRODUCTION

One of the primary effects of insulin is to increase the rate of glucose transport and utilization in muscle and adipose tissue (for review see [1]). Recently it has been demonstrated that catecholamines can oppose this effect of insulin on muscle [2]. However, studies of effects of catecholamines on glucose utilization in adipose tissue have given conflicting results. Several authors have reported that catecholamines actually mimic the effect of insulin on glucose transport and utilization in isolated adipose tissue and adipocytes [3–5].

Recently it has been reported that glucagon can antagonize the effect of insulin on glucose transport in adipocytes [6]. However, the effect of glucagon was only observed if adenosine, which is produced spontaneously by isolated adipocytes [7], was prevented from accumulating in the incubation medium by addition of adenosine deaminase.

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Therefore it was of interest to investigate the effects of catecholamines on glucose transport in adipocytes, in the presence and in the absence of adenosine deaminase. The results demonstrate that catecholamines can inhibit insulin-stimulated 2-deoxyglucose uptake, but only in the presence of adenosine deaminase. This suggests that adenosine released from the cells attenuates the ability of catecholamines to inhibit insulin-stimulated glucose transport, and that adenosine and catecholamines are regulators of insulin action in adipose tissue.

2. MATERIALS AND METHODS

Porcine insulin was generously given by Dr Ronald Chance of Eli Lilly (Indianapolis, IN). Bovine serum albumin (BSA) (fraction V) was purchased from Armour Pharmaceuticals (Phoenix, AZ). Collagenase was from Worthington Biochemicals (Freehold, NJ). L-[1-^{3}H]glucose and 2-deoxy-D-[1-^{3}H]glucose were from New England Nuclear (Boston, MA). 2-Deoxy-D-glucose, N-2-hydroxyethylpiperazine-N^1-2-ethanesulphonic

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

acid (HEPES), *N*-tris[hydroxymethyl]methyl glycine (Tricine), epinephrine, norepinephrine, isoproterenol, propranolol and adenosine deaminase (EC 3.5.4.4) were from Sigma (St Louis, MO). Phentolamine was from CIBA (Summit, NJ). Adenosine deaminase was supplied as a suspension in 3.2 M $(NH_4)_2SO_4$. Before use the enzyme was dialyzed against 2 × 500 vol. of 154 mM NaCl, 10 mM HEPES (pH 7.4) to remove $(NH_4)_2SO_4$. Male Sprague–Dawley rats weighing 190–230 g were purchased from Charles River (Cambridge, MA).

Adipocytes were isolated from epididymal fat pads as in [8]. Cells were filtered through 250 μm nylon mesh, centrifuged at 25 × *g* for 90 s and washed 3-times in the incubation buffer (see below). The cells (~2 × 10^5/ml) were then incubated with shaking for 1 h at 37°C in a buffer containing 137 mM NaCl, 5 mM KCl, 4.2 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 10 mM HEPES, 10 mM Tricine and BSA (10 mg/ml) (pH 7.4) and additions as described in the text and figure legends.

At the end of the incubation period glucose transport was measured by the 2-deoxyglucose uptake method [9] as follows: 2-Deoxy-D-[1-^{3}H]glucose (spec. act. 1.6 mCi/mmol) was added to a final concentration of 0.1 mM. The assays were terminated 3 min later by transferring 200-μl samples of the cell suspension to plastic microtubes containing silicone oil (100 μl). The tubes were centrifuged for 30 s in a Beckman 'Microfuge', and the assay was considered terminated when centrifugation began. The tubes were cut through the oil layer with a razor blade, and the radioactivity in the cell pellet was measured in a liquid scintillation counter. In each experiment, similar incubations were performed using L-[1-^{3}H]glucose in place of 2-deoxyglucose to determine the amount of sugar trapped in the extracellular water space of the cell layer. All data have been corrected for this factor.

Adipocytes were counted by a modification of Method III in [10]. The cells were fixed in 2% osmium tetroxide in 50 mM collidine buffer (made isotonic with NaCl) for 24 h at 37°C, and then counted in a Coulter counter, model ZB. All results have been normalized to a cell concentration of 2.5 × 10^5 cells/ml.

3. RESULTS

3.1. *Effects of catecholamines on glucose transport*

In the absence of adenosine deaminase, epinephrine, norepinephrine and isoproterenol had no effect on either basal or insulin-stimulated 2-deoxyglucose uptake (fig.1A). In the presence of

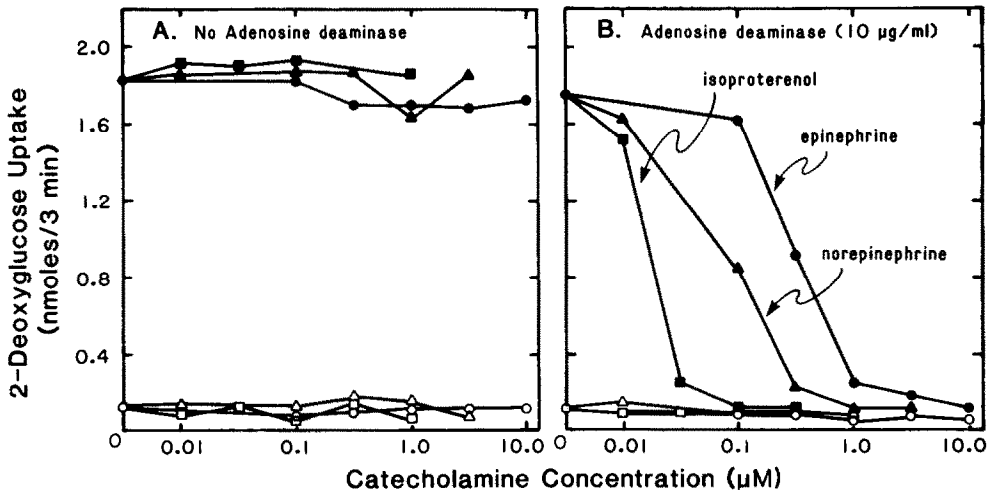

Fig.1. Effects of catecholamines on basal and insulin-stimulated glucose transport in the absence (A) or in the presence (B) of adenosine deaminase. Adipocytes were incubated at 37°C without (open symbols) or with (closed symbols) insulin (25 ng/ml) plus the indicated concentrations of epinephrine (○,●), norepinephrine (△,▲) or isoproterenol (□,■). After 1 h, 2-deoxyglucose uptake was measured as described in section 2.

adenosine deaminase however, all 3 catecholamines completely inhibited the effect of insulin on 2-deoxyglucose uptake (fig.1B). There was no effect of catecholamines on basal uptake rates in the presence of the enzyme. Half-maximally effective concentrations of the catecholamines in the presence of the enzyme were about 0.3 μM, 0.1 μM and 0.02 μM for epinephrine, norepinephrine and isoproterenol, respectively. Thus catecholamines inhibit the effect of insulin on glucose transport, but only in the presence of adenosine deaminase.

The effect of isoproterenol on insulin-stimulated 2-deoxyglucose uptake was not observed when N^6-phenylisopropyl adenosine (an adenosine analogue which is not deaminated by adenosine deaminase) was included together with the enzyme (fig.2). Similarly, epinephrine and norepinephrine did not inhibit the insulin-stimulated uptake rates when the analogue was included together with the enzyme (not shown). This suggests that in the presence of adenosine deaminase it is the lowered adenosine level, rather than the presence of the enzyme itself, which allows the catecholamines to inhibit the insulin effect.

3.2. *Effects of α- and β-blockers*

In order to investigate which class of adrenergic receptors mediates the effect of catecholamines on 2-deoxyglucose uptake, the adrenergic blocking agents phentolamine (α-blocker) and propranolol (β-blocker) were utilized. Cells were incubated with propranolol or phentolamine for 10 min prior to addition of hormones. Phentolamine did not affect the ability of epinephrine (10 μM) to inhibit insulin-stimulated glucose transport in the presence of adenosine deaminase (fig.3). However, propranolol (10 μM) completely blocked the effect of the catecholamine. Similarly, the effects of 1 μM norepinephrine or 0.5 μM isoproterenol were prevented by the β-blocker but not the α-blocker (not shown). Therefore this effect of catecholamines seems to be mediated via β-adrenergic receptors.

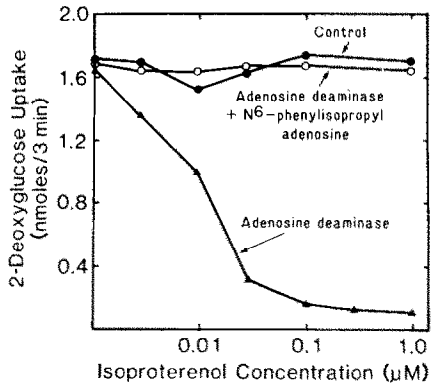

Fig.2. Effect of isoproterenol on insulin-stimulated glucose transport in the presence or absence of adenosine deaminase and N^6-phenylisopropyl adenosine. Adipocytes were incubated at 37°C with insulin (25 ng/ml) plus: (●) no further additions; (▲) adenosine deaminase (10 μg/ml); (○) adenosine deaminase (10 μg/ml) and N^6-phenylisopropyl adenosine (0.1 μM). After 1 h, 2-deoxyglucose uptake was measured as described in section 2.

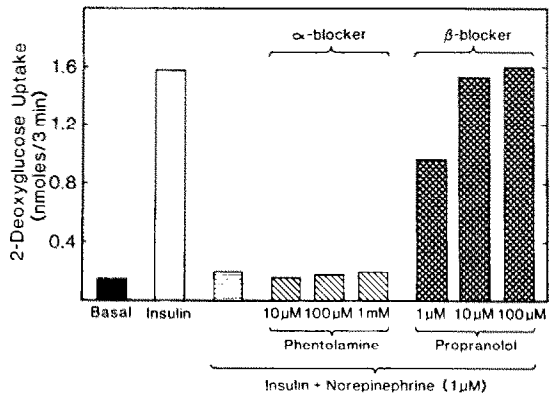

Fig.3. Effects of adrenergic blocking agents on the ability of epinephrine to inhibit insulin-stimulated glucose transport in the presence of adenosine deaminase. Adipocytes were incubated at 37°C with adenosine deaminase (10 μg/ml) plus phentolamine or propranolol as indicated. After 10 min insulin (25 ng/ml) and epinephrine (10 μM) were added as indicated. After a further 1 h, 2-deoxyglucose uptake was measured as described in section 2.

4. DISCUSSION

2-Deoxyglucose is taken up by adipocytes by the D-glucose transport system and provides a good index of the rate of glucose transport [9]. The results presented in this report demonstrate that catecholamines inhibit insulin-stimulated 2-deoxyglucose uptake by adipocytes in the presence (but not absence) of adenosine deaminase. This catecholamine effect is not observed in the presence of the enzyme plus

N^6-phenylisopropyl adenosine (an adenosine analogue which is not deaminated by adenosine deaminase). Furthermore, adipocytes are known to release small amounts of adenosine into the incubation medium [7]. Taken together these observations strongly suggest that catecholamines can antagonize the effect of insulin on glucose transport and that adenosine, released from the cells, can block this effect of catecholamines.

It has previously been demonstrated that adenosine deaminase can decrease the insulin sensitivity of glucose utilization [11] and glucose oxidation [12] in adipocytes. This has recently been shown to result, at least partly, from a decrease in the insulin sensitivity of the glucose transport system [6]. Adenosine deaminase does not, by itself, alter the response of glucose transport to a maximally effective concentration of insulin, 25 ng/ml [6]. Insulin has been used at a maximal concentration (25 ng/ml) throughout the current studies, which explains why adenosine deaminase by itself did not significantly alter the rate of glucose transport (see fig.1). However, in the presence of adenosine deaminase, catecholamines inhibit the effect of this maximally effective concentration of insulin. Thus it appears that catecholamines and adenosine can interact to regulate the maximum response of glucose transport to insulin.

The concentration of adenosine in adipose tissue in vivo is unknown. Similarly, the change in adenosine concentration produced by adenosine deaminase in the current studies is not known. Therefore it is difficult to assess the possible physiological significance of these findings. However, the current findings result from removal of adenosine produced spontaneously by isolated adipocytes. Therefore the cells are capable of producing sufficient adenosine to modify their response to insulin and catecholamines. This suggests that adenosine and catecholamines are regulators of insulin action in adipose tissue in vivo.

ACKNOWLEDGEMENTS

I am grateful to Dr J.M. Olefsky for invaluable advice and encouragement and to Dr S. Marshall for reviewing the manuscript.

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