The effect of N^6-phenylisopropyladenosine on the regulation of fat cell hexose transport, glucose oxidation and fatty acid release by insulin and catecholamines

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N^6-Phenylisopropyladenosine was employed in the absence of endogenous adenosine to explore the influence exerted by the R-site over the antagonistic interaction of insulin and catecholamines on several parameters of fat cell metabolism. When no hormones were present, N^6-phenylisopropyladenosine had little or no effect; however, the nucleoside potentiated insulin inhibition of catecholamine-stimulated events, such as lipolysis, and, conversely, diminished or blocked catecholamine inhibition of insulin-stimulated processes, such as 2-deoxyglucose uptake, glucose oxidation and esterification, even under conditions where N^6-phenylisopropyladenosine, alone, was ineffective in reversing catecholamine actions.

N^6-*phenylisopropyladenosine* *Fat cell metabolism* *Insulin* *Catecholamine* *Modulation*

1. INTRODUCTION

Insulin and catecholamines generally exert opposing actions on fat cell metabolism. The antilipolytic action of insulin is well documented (review [1]); however, the hormone has little effect when glycerol release from fat cells is stimulated by 10^{-6} M norepinephrine, in the presence of adenosine deaminase [2–4]. Adenosine and R-site analogues, such as N^6-phenylisopropyladenosine (PIA), are also ineffective in inhibiting lipolysis stimulated by 10^{-6} M norepinephrine. A combination of insulin and either adenosine or PIA, however, strongly inhibits catecholamine-stimulated glycerol release [2–4]. This suggests a role for the R-site, under certain circumstances, in modulating the antagonistic interaction of insulin and catecholamines on lipolysis. These data indicate that the R-site exerts a similar influence over the opposing actions of insulin and catecholamines on fat cell hexose transport, glucose oxidation and esterification.

2. EXPERIMENTAL

Crude *Clostridium histolyticum* collagenase (EC 3.4.24.3, lot 37C-6860), bovine serum albumin (BSA) (Cohn fraction V; lot 88C-0252), epinephrine, norepinephrine, insulin (bovine), and adenosine deaminase (ADA) were obtained from Sigma Chemicals (St Louis MO). All radiochemicals were purchased from Amersham (Arlington Heights IL). N^6-(Phenylisopropyl)adenosine was a gift from Dr Harald Stork of Boehringer (Mannheim).

2.1. *Treatment of rats and preparation of fat cells*

Male Wistar rats (180–200 g) were allowed free access to laboratory chow until the time of sacrifice. Rats were stunned by a blow to the head and killed by cervical dislocation. The epididymal fat pads were excised, washed in warm saline (37°C), minced and digested in a bicarbonate-buffered medium (KRB) (pH 7.4) containing 3% bovine serum albumin (BSA), glucose (2 mg/ml)

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and collagenase (1 mg/ml) [5]. Liberated cells were filtered through a nylon mesh, washed twice in glucose-free medium, and resuspended in the buffer. Fat cell DNA was measured following the method in [6].

2.2. *Measurement of lipolysis*

Cells were incubated in 2 ml KRB containing 3% BSA at 37°C in a Dubnoff metabolic incubator. At the end of the incubation, aliquots of the medium were removed from beneath the floating layer of fat cells for the determination of glycerol and non-esterified fatty acids (NEFA). Glycerol, in deproteinized and neutralized samples, was assayed enzymatically by fluorimetric method in [7]. Aliquots of medium, after extraction in Dole's mixture [8], were assayed for NEFA, employing the colorimetric determination in [9].

2.3. *Conversion of [^{14}C]glucose to $^{14}CO_2$ and lipids*

Determination of [^{14}C]glucose oxidation has been described in [10].

2.4. *Glucose transport studies*

Glucose transport was measured as in [11] using either D-3-*O*-methyl[^{14}C]glucose or 2-D-deoxy[^{3}H]glucose as substrate. A concentrated fat cell suspension (50 μl) was preincubated with additions for 15 min in Eppendorf microcentrifuge tubes. The reaction was initiated by rapid addition of 20 μl labeled sugar (3 nmol) to the fat cell suspension. Transport was terminated at the desired time by addition of buffer (0.4 ml) containing 0.3 mM phloretin. To correct for extracellular trapping of water, uptake was measured in parallel incubations in the presence of 50 μM cytochalasin B and subtracted from the other values obtained. Cells were then separated from the buffer by the oil-flotation technique [12] and removed with a piece of absorptive material into a scintillation vial and counted for ^{14}C- or ^{3}H-radioactivity.

Radioactivity was measured in Aquasol using a Packard Tricarb Liquid Scintillation Spectrometer (Model 574). Quenching corrections were applied using the channel's ratio method.

3. RESULTS AND DISCUSSION

The role of adenosine in modulating hormone action on fat cell metabolism was studied in rat adipocytes. The R-site analogue of adenosine, PIA, in the absence of endogenous adenosine, was used to explore this relationship. The antagonistic interaction of catecholamines, such as norepinephrine and epinephrine, and the polypeptide hormone, insulin, on hexose transport, glucose oxidation, glycerol release and NEFA re-esterification served as the model of hormone action. A role for adenosine in fat cell metabolism has been suggested, on one or more parameters [13–16]. This work aimed to extend the study to several parameters, under identical conditions which control for the level of endogenous adenosine in the cell [17,18]. Maximally effective concentrations of PIA, insulin and catecholamines were employed throughout.

A combination of epinephrine and insulin, in the presence of adenosine deaminase, stimulates 3-*O*-methylglucose (3-OMG) uptake 2–3-fold above basal values (fig.1). Inclusion of the R-site analogue, PIA, had no effect on 3-OMG transport induced by these two hormones (fig.1). In contrast, PIA does reverse the effects of epinephrine in inhibiting basal and insulin-stimulated 2-deoxyglucose (2-DG) transport in the presence of adenosine deaminase (table 1). PGE_1, nicotinic acid and 2-deoxycoformycin, an inhibitor of adenosine deaminase, also reverse the effects of epinephrine on 2-DG transport (not shown).

These results: (1) support the concept of different regulatory processes for 2-DG and 3-OMG transport as suggested in [19,20]; (2) strongly implicate a role for the R-site in modulation of hexose transport as modified by hormonal treatment.

PIA elicited a pattern of responses on [1-^{14}C]glucose oxidation which was essentially similar to its effects on 2-DG uptake. The R-site analogue exerted its greatest effect on the combination effect of insulin plus norepinephrine (fig.2). In the presence of adenosine deaminase, norepinephrine strongly depressed glucose oxidation stimulated by insulin. However, when PIA was added, norepinephrine actually slightly enhanced the stimulation of glucose conversion to CO_2 induced by insulin. Also the high C_1/C_6 ratio (ratio of glucose carbon 1 to glucose carbon 6 ox-

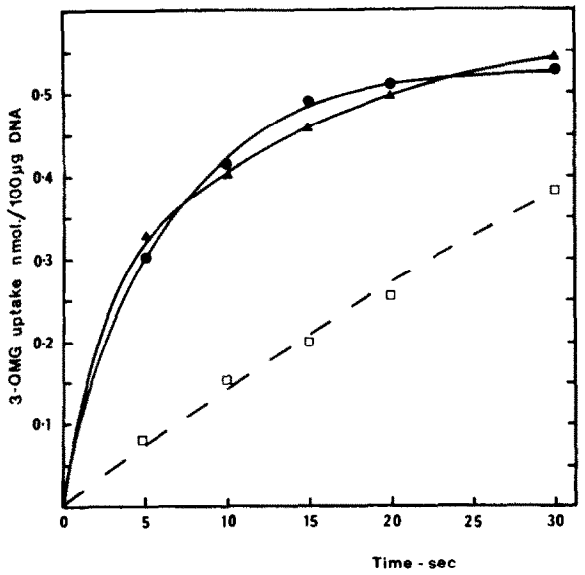

Fig.1. The effect of PIA on 3-OMG uptake in the presence of insulin and epinephrine. Fat cells were incubated with epinephrine (1 μM) and insulin (3.3 nM) in the presence (●) and absence (▲) of PIA (0.1 μM) for 15 min at 37°C in KRB (3% BSA) containing ADA (2 μg/ml). Uptake was initiated by the forceful addition of 20 μl buffer containing 3-*O*-methyl[^{14}C]glucose (3 nmol, 0.2 μCi) and terminated at the indicated times as in section 2. The results are presented as the means of 3 duplicated expts. The discontinuous line represents 3-OMG uptake in the absence of other additions (+ADA).

Table 1

The effect of PIA, epinephrine and insulin on 2-DG uptake in isolated fat cells

Addition	(−)	(+)PIA
None	2.046 ± 0.321	2.913 ± 0.328
Insulin	5.599 ± 0.532	5.711 ± 0.742
Epinephrine	1.011 ± 0.271	2.480 ± 0.381
Insulin + epinephrine	3.647 ± 0.675	6.064 ± 0.626

The conditions employed for the measurement of hexose transport are described in section 2. Insulin (3.3 nM), epinephrine (1 μM) and PIA (0.1 μM) were included, where indicated, together with adenosine deaminase (2 μg/ml). The results are expressed in nmol/100 μg. DNA and represent the means ± SEM of 3 duplicated expts

idized), indicative of the quantity of glucose processed via the pentose phosphate shunt, observed with insulin, was lowered by norepinephrine; the lowering effect of catecholamine on the insulin-enhanced C_1/C_6 ratio was partially reversed by PIA.

Modulation by PIA of the antagonistic interaction of catecholamine and insulin on glycerol release was reported in [2–4], and these results extend those findings (fig.3). Our results indicate that not only is insulin inhibition of catecholamine-stimulated lipolysis influenced by PIA, but also the efficacy with which insulin stimulates re-esterification of liberated non-esterified fatty acids (NEFA) (fig.3). PIA slightly stimulated the removal by fat cells of NEFA from the incubation medium. This effect of PIA was potentiated in the presence of glucose (10 mM). Insulin (3.3 nM) also slightly stimulated NEFA removal from the incubation medium and again this effect of the hormone was enhanced by

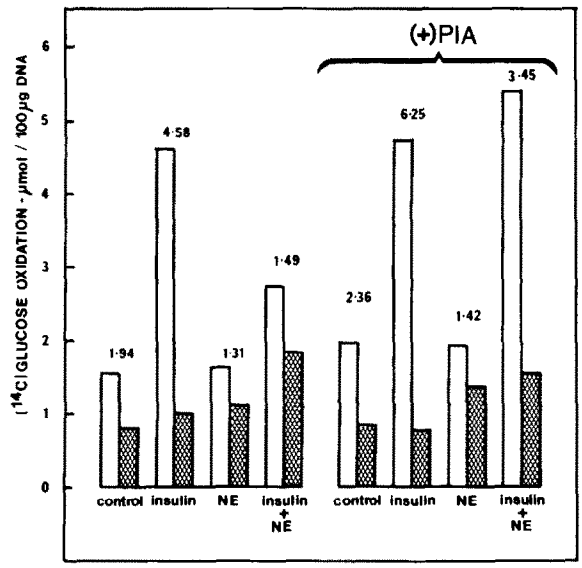

Fig.2. The effect of insulin, norepinephrine and PIA on [1-^{14}C]glucose and [6-^{14}C]glucose conversion to $^{14}CO_2$. Fat cells were incubated in KRB containing BSA (3%), ADA (2 μg/ml) with [1-^{14}C]glucose (open bars) or [6-^{14}C]glucose (shaded bars) (5 mM, 0.2 μCi) for 1 h at 37°C. Insulin (3.3 nM), norepinephrine (1 μM) and PIA (0.1 μM) were included where indicated. The values appearing above the histograms are the C_1/C_6 ratio. The results are presented as means of 3 duplicated expts.

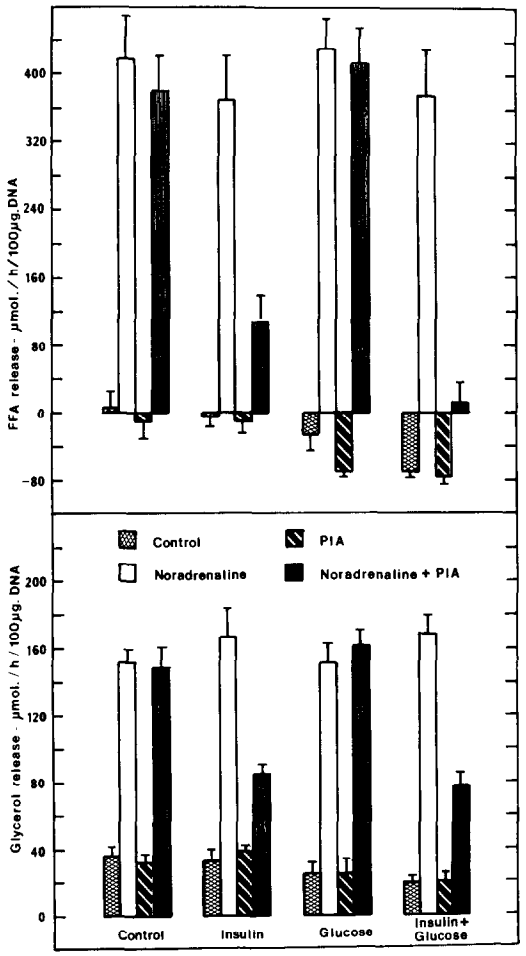

Fig.3. The effect of insulin and glucose on basal and epinephrine-stimulated glycerol and NEFA release in the presence and absence of PIA. Fat cells were incubated for 1 h at 37°C in KRB containing BSA (3%), ADA (2 µg/ml) and, where indicated, norepinephrine (1 µM), glucose (10 mM), insulin (3.3 nM) and PIA (0.1 µM). For both glycerol and NEFA, the zero time value (i.e., the value of samples taken immediately after addition of suspended cells to the incubation medium) was subtracted from the final value to give the release rate per hour. The BSA used (Sigma, lot 88C-0254) contained 14.5 µmol NEFA/g (molar ratio NEFA : BSA 0.87). The results represent the means ± SEM of 3 duplicated expts.

glucose. PIA and insulin, when added alone, had little effect on norepinephrine-stimulated NEFA liberation; however, as with glycerol release, a combination of the two agents elicited a strong inhibitory effect (fig.3). In the presence of glucose, the inhibition of norepinephrine-stimulated NEFA release caused by PIA and insulin was greatly enhanced, although the sugar had no measurable effect on glycerol release. Glucose probably increased re-esterification of released NEFA by furnishing a source of α-glycerophosphate.

On several parameters of fat cell metabolism the pattern of responses elicited by PIA is similar. PIA is generally only weakly effective in the absence of hormones; however, the nucleoside potentiates insulin inhibition of catecholamine-stimulated events, such as lipolysis, and, conversely, reduces or blocks catecholamine inhibition of insulin stimulated processes, such as 2-DG transport, [1-^{14}C]glucose oxidation and esterification, even under conditions where PIA alone is ineffective in reversing catecholamine actions. The mechanism by which PIA modulates the antagonistic interaction of insulin and catecholamines awaits further study.

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