THE STIMULATION OF [1-^{14}C]GLUCOSE OXIDATION IN ISOLATED FAT CELLS BY N^6-METHYLADENOSINE

An effect independent of cyclic AMP

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1. Introduction

Adenosine potentiates basal and insulin-stimulated [1-^{14}C]glucose oxidation in fat cells [1]. Although the effect of the nucleoside may be mediated by a mechanism independent of that by which adenosine inhibits lipolysis [1], it is now generally accepted that the conversion of [1-^{14}C]glucose to $^{14}CO_2$ is secondary to an inhibition of cyclic AMP synthesis [2,3]. This is supported by the findings that other inhibitors of cyclic AMP accumulation, such as prostaglandin E_1 and nicotinic acid mimic the potentiation, by adenosine, of insulin action [2,3]. In addition, a good inverse correlation has been reported between intracellular cyclic AMP concentrations and [1-^{14}C]glucose oxidation, under conditions in which glucose transport was made rate limiting [4,5]. The greatly diminished insulin response when endogenous adenosine is removed from the incubation medium with adenosine deaminase has been attributed to the increased intracellular cyclic AMP [2,3,6]. Although insulin stimulates [1-^{14}C]glucose conversion to $^{14}CO_2$ by a mechanism which is not mediated by cyclic AMP, the hormone is a weak effector of glucose oxidation in the presence of high levels of the cyclic nucleotide [2,3,6].

Results presented here indicate that the adenosine analogue, N^6-methyladenosine, strongly stimulates [1-^{14}C]glucose oxidation by a cyclic AMP-independent mechanism, suggesting that more than one mechanism may be involved in the action of adenosine on fat cell glucose metabolism.

2. Materials and methods

Crude *Clostridium histolyticum* collagenase (lot 40C190) was purchased from Worthington Biochemical Corp. (Freehold NJ). Bovine serum albumin (Cohn fraction V), noradrenaline, adenosine deaminase and other enzymes and cofactors were supplied by Sigma Chemical Co. (St Louis MO). D-[1-^{14}C]Glucose and cyclic AMP kit, TRK-432, were obtained from the Radiochemical Centre (Amersham, Bucks.). P. L. Biochemicals (Milwaukee WI) supplied N^6-methyladenosine and 2′5′-dideoxyadenosine. N^6-Phenylisopropyladenosine (Boehringer, Mannheim) was a generous gift from Dr J. Cadenas of Farmacéuticos Lakeside (México). All other reagents were of the highest grade available.

Male Wistar rats (200–230 g) were allowed free access to laboratory chow until the time of sacrifice. Adipocytes were prepared using the method in [7], with minor modifications as in [8]. After washing the cells 3 times, the cells were suspended in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin. Aliquots (0.5 ml) were added to polythene scintillation vials, containing various additions, to bring the final incubation volume to 2 ml in all studies except those in which cyclic AMP was assayed, where the final volume was 1 ml. [1-^{14}C]Glucose oxidation was measured essentially as in [8] with $^{14}CO_2$ being trapped in suspended wells containing 0.2 ml hyamine hydroxide (Amersham/Searle). Radioactivity was counted in 10 ml of a toluene-based scintillation fluid containing 2,5-*p*-phenylene-bis-(5-phenyloxazole) (PPO) (5 g/l) and 1,4-bis 2-(4-methyl-5-phenyloxazolyl) benzene (POPOP) (0.1 g/l), with a counting

efficiency of 84%. Conversion of [1-^{14}C]glucose to $^{14}CO_2$ was expressed in the form μmol/100 μg DNA.

Glycerol was measured enzymatically according to [9] and fat cell DNA following [10].

Fat cell cyclic AMP was measured essentially as in [5]. After a 10 min incubation of fat cells with lipolytic agents and separation of cells from medium, using the oil-floatation method in [11], little (<3%) extracellular cyclic AMP was observed and, for this reason, the cyclic nucleotide was routinely assayed in cells plus medium.

3. Results and discussion

Work with analogues of adenosine has demonstrated the existence of two adenosine receptors, R and P, associated with fat cell adenylate cyclase [12,13]. The P-site, with strict structural specificity with respect to the purine moiety of the nucleoside is postulated to be located on the intracellular surface of the plasma membrane and appears to be of only pharmacological importance [12,13]. The externally located R-site (now designated R_1 [14]) with its requirement for the integrity of the ribose moiety, is believed to be the physiologically important receptor in mediating adenosine actions [12–14]. N^6-Phenylisopropyladenosine (PIA), the potent R-site agonist, inhibits hormone-stimulated cyclic AMP accumulation and lipolysis at sub-μM levels [6]. The inhibition of adenylate cyclase by PIA is dependent upon the presence of inhibitory concentrations of GTP in the incubation medium [13]. In contrast, the strong P-site effector, dideoxyadenosine (DDA), which exhibits no such GTP dependence in its action on adenylate cyclase, inhibits hormone-stimulated cyclic AMP synthesis only in the μM range and has no effect on lipolysis [6].

In fig.1, the effect of R-site active analogues PIA and N^6-methyladenosine and the P-site analogue, DDA, on [1-^{14}C]glucose conversion to $^{14}CO_2$ is presented. An appreciable increase in the formation of $^{14}CO_2$ is seen at 20 μM N^6-methyladenosine, with a maximal (>2-fold) increase observed at 100 μM. Neither PIA nor DDA induced a measurable rise in the oxidation of [1-^{14}C]glucose over a wide concentration range. The findings, with respect to PIA action, agree with [2,3], where, under similar conditions (30–50 mg triglyceride/incubation), no stimulatory effect was observed. It is important to note that a significant effect of adenosine, PIA and other inhibi-

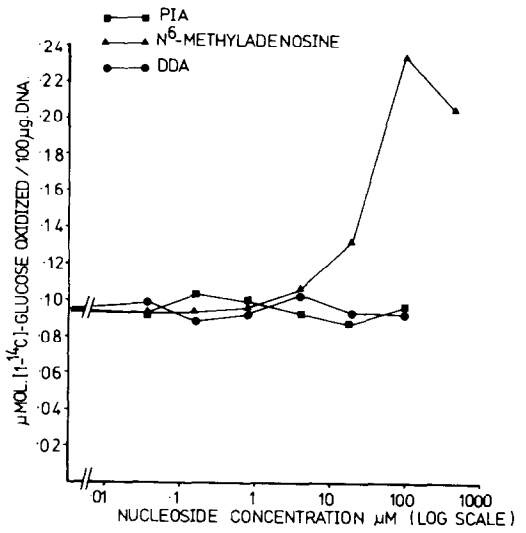

Fig.1. The effect of adenosine analogues on [1-^{14}C]glucose oxidation to $^{14}CO_2$. Fat cells (0.75–1.00 μg DNA/ml) were incubated for 45 min in the presence of 0.5 mM (0.2 μCi) [1-^{14}C]glucose. (For details of the incubation see section 2.) Adenosine deaminase was present at 2 μg/ml. The results are expressed as the means of 3 paired expt.

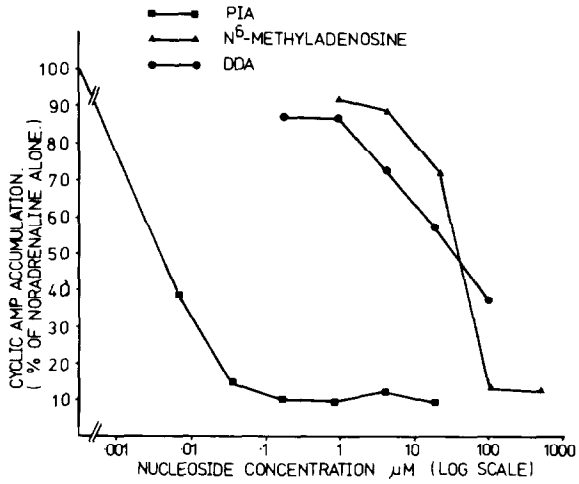

Fig.2. The effect of adenosine analogues on cyclic AMP accumulation stimulated by noradrenaline. Fat cells (0.75–1.00 μg DNA/ml) were incubated for 10 min in 1 ml incubation medium containing noradrenaline (1 μM) and adenosine deaminase (2 μg/ml) plus the stated concentrations of the adenosine analogues. Basal cyclic AMP levels in the absence of adenosine deaminase were 1.0–1.5 nmol/100 μg DNA. The results represent the means of 3 paired expt.

tors of cyclic AMP accumulation, such as prostaglandin E_1 and nicotinic acid, on glucose oxidation is difficult to demonstrate unless a relatively, low fat cell concentration is used [1–3]. Under these conditions, it is argued, the low endogenous adenosine concentration, in dilute cell suspensions, results in higher intracellular cyclic AMP levels, the lowering of which leads to an increased conversion of [1-^{14}C]glucose to $^{14}CO_2$.

The effect of adenosine analogues on noradrenaline and adenosine deaminase-stimulated cyclic AMP accumulation is presented in fig.2. PIA is $>10^3$-times more potent as an inhibitor of cyclic AMP accumulation than DDA and N^6-methyladenosine. The strong effect of N^6-methyladenosine, observed in the 20–100 μM range, probably reflects its action as a weak R-site effector [13]. Although both R-site active analogues exhibit a strong inhibitory effect on hormone-stimulated cyclic-AMP synthesis, only N^6-methyladenosine increases [1-^{14}C]glucose conversion to $^{14}CO_2$ under these conditions, seemingly excluding the participation of the R-site and the cyclic nucleotide in the action of N^6-methyladenosine on glucose oxidation.

The actions of N^6-methyladenosine on cyclic AMP accumulation and [1-^{14}C]glucose oxidation in the presence of adenosine deaminase and noradrenaline were tested over a narrower range (20–100 μM) in order to investigate how closely the fall in the cyclic nucleotide concentration matched the increased production of $^{14}CO_2$ (fig.3). In addition, the effect of the analogue on glycerol release was measured. The increase in [1-^{14}C]glucose oxidation over this concentration range is roughly linear with a clear increment observable at as low as 20 μM nucleoside. Although a sharp decrease in the cyclic AMP concentration is seen over the same range, the levels of the cyclic nucleotide remain high until N^6-methyladenosine reaches 60 μM. It is worth noting that, in contrast to insulin which is a poor stimulator of glucose oxidation in the presence of elevated cyclic AMP levels [2,3,6], N^6-methyladenosine enhances $^{14}CO_2$ production from [1-^{14}C]glucose when concentrations of the cyclic nucleotide are elevated to the extremely high levels observed in the presence of adenosine deaminase and noradrenaline. The analogue has only a slight inhibitory effect on lipolysis in the concentration range employed.

The above results indicate that the action of N^6-methyladenosine on glucose metabolism is unrelated to the P- and R-adenosine receptors and seems to be independent of changes in cyclic AMP levels,

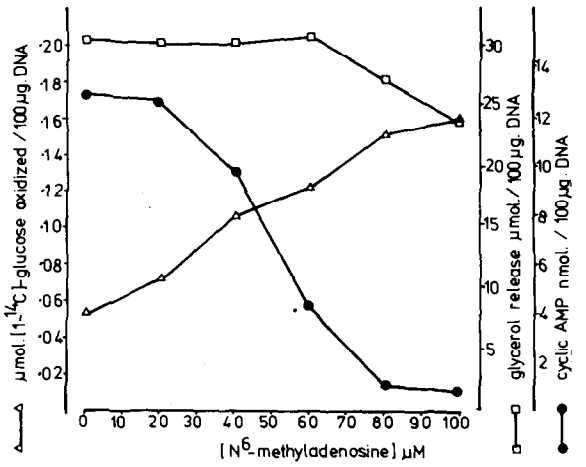

Fig.3. The effect of N^6-methyladenosine on [1-^{14}C]glucose oxidation, cyclic AMP accumulation and glycerol release in the presence of noradrenaline and adenosine deaminase. Fat cell was 0.75–1.00 μg DNA/ml. Noradrenaline (1 μM) and adenosine deaminase (2 μg/ml) were present throughout. Cells were incubated in 2 ml total vol. for 45 min, in the case of the lipolysis and glucose oxidation studies, and in 1 ml for 10 min, in the case of the cyclic AMP studies. The results are reported as the means of 3 paired expt.

although an effect on a small unmeasurable pool of the cyclic nucleotide cannot be ruled out. Although further work will have to be undertaken to clarify the mechanism of action of N^6-methyladenosine, the results raise the intriguing possibility that more than one mechanism may be involved in the action of adenosine on glucose metabolism. Whether a receptor, unrelated to the P- and R-sites, is involved, or metabolism of the nucleoside is an important first step is, at the moment, being investigated.

Acknowledgements

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