Adenosine effects upon insulin action on lipolysis and glucose transport in human adipocytes

Lesley Heseltine, Judith M. Webster and Roy Taylor

Human Metabolism Research Centre, Medical School, University of Newcastle upon Tyne, NE2 4HH UK

Received 4 August 1994; accepted 30 November 1994

Abstract

The dose response effect of a new adenosine analogue, GR79236 (N-[1S trans-2-hydroxycyclopentyl] adenosine) upon insulin sensitivity was examined in human adipocytes. The influence of adenosine upon insulin sensitivity for suppression of lipolysis and stimulation of glucose transport was examined. Removal of adenosine by use of adenosine deaminase stimulated lipolysis to the same extent as did 10^{-4}M noradrenaline. GR79236 brought about dose dependent inhibition of lipolysis with half-maximal effect at 11.3 \pm 7.8 \times 10^{-4} M. When lipolysis was stimulated by noradrenaline alone the subsequent inhibition of lipolysis brought about by GR79236 was significantly greater than that of insulin. To examine adenosine effects on the insulin signalling pathway separately from those on lipolysis, the insulin sensitivity of glucose transport was examined. Removal of adenosine brought about a small but significant increase in the concentration of insulin required for half-maximal stimulation of glucose transport. Adenosine agonists offer promise as new agents for the modulation of metabolism in diabetes and other states of insulin resistance. (Mol Cell Biochem: 144:147-151, 1995)

Key words: adenosine, adenosine agonist, human adipocyte, lipolysis, insulin sensitivity

Introduction

Adipose tissue lipolysis is a crucial rate limiting step for control of plasma non-esterified fatty acid concentrations and thus substrate for oxidation by liver and muscle. Modulation of lipolysis offers the potential of enhancing glucose metabolism via the glucose-fatty acid cycle. This could bring about practical benefit in non-insulin dependent diabetes, a state characterised by high circulating fatty acid levels, hyperglycaemia and decreased basal rates of glucose oxidation [1, 2]. In NIDDM, adipocytes show decreased sensitivity to insulin [3, 4]. Human obesity is also characterised by raised plasma fatty acid levels and decreased glucose oxidation, and both decreased numbers of adenosine receptors on adipocytes and decreased sensitivity to adenosine have been reported [5].

Adenosine has been recognised as an anti-lipolytic agent for several years and has recently been shown to modulate the glucose-fatty acid cycle in heart muscle [6]. Effects of adenosine removal upon lipolysis and glucose transport in rat adipocytes have been described [7, 8]. The antilipolytic effect of A1 adenosine receptor agonists in the presence of adenosine deaminase has been reported [9]. However, the dose response effects of the A1 receptor agonists on human adipocytes and the effects of adenosine removal upon lipolysis and glucose transport have not been fully characterised.

The present studies were designed to elucidate the dose response effects of the new highly selective A1 receptor agonist GR79236 (N-[1S trans-2-hydroxycyclopentyl] adenosine) [10], to document the effect of adenosine agonists upon measured insulin sensitivity of lipolysis and to determine the effect upon human adipocyte glucose transport of removing endogenous adenosine.

Materials and methods

Chemicals

Human serum albumin was obtained from Hoechst-Behring UK Ltd., silicone oil (density 0.97 g/ml) from Dow Corning...
Corporation, UK, and crude collagenase (type II), noradrenaline ((-)-Norepinephrine) and phloretin all from Sigma, UK. Uniformly labelled 3-O-methyl-D-[U-14C] glucose (4.7 GBq/mmol) was purchased from the Radiochemical Centre, Amersham, UK, adenosine deaminase from Boehringer Mannheim Limited, UK, and scintillation fluid (Optiphase Hi-safe II) from LKB, UK. Crystalline monocomponent porcine insulin was a gift from Novo and GR79236 N-[1S trans-2-hydroxycyclopentyl]adenosine was provided by Glaxo Group Research Limited, UK.

**Cell isolation**

Subcutaneous abdominal adipose tissue was obtained with prior consent from patients with no known metabolic disorder who were undergoing elective surgery for hysterectomy or gastroenterological reasons. Hence, cells were obtained from subjects who fasted overnight. The procedure was approved by the Newcastle Joint Hospital and University Ethical Committee. Adipocytes were prepared by the method of Pedersen et al. [11]. The adipose tissue was finely chopped and incubated for 90 min at 37°C in a HEPES buffer (pH 7.4) containing human serum albumin (25 g/l) and collagenase (0.5 g/l). The isolated adipocytes were subsequently washed in a HEPES buffer containing human serum albumin (50 g/l) either with glucose (5 mM) for lipolysis studies or without glucose for glucose uptake studies.

**Measurement of lipolysis**

Lipolysis was assessed by glycerol release from the adipocytes [12]. The cell suspension was adjusted to allow a final lipocrit of 5% after addition of the hormones and/or adenosine deaminase and/or adenosine agonists or buffer alone. An aliquot of the cell suspension (50 µl) was taken for measurement of cell diameters. The diameters of 150 adipocytes were measured at 200-fold magnification using an eyepiece micrometer. Mean cell surface area, volume and number per tube were calculated from cell diameters as above. Results of initial 3-O-methyl-D-[U-14C] glucose transport in the adipocytes were expressed as pmol 3-O-methyl-D-[U-14C] glucose taken up per 10 cm² cell membrane surface in the 5 sec incubation time.

**Statistics**

All results are presented as mean ± SE and difference between means was tested using Student’s paired t test.

**Results**

Lipolysis was stimulated to a similar extent by 10⁻⁷ M noradrenaline alone (3.0-fold to 167 ± 27 nmols/10⁶ cell per 90 mins) or removal of adenosine by use of 1 unit/ml ADA (2.3-fold to 129 ± 22 nmols/10⁶ cells per 90 min) (Table 1). When both noradrenaline and ADA were present no further stimulation of lipolysis was seen. In this system the isolated human adipocytes were exquisitely sensitive to insulin, with maximum suppression of lipolysis observed at 10⁻⁸ M insulin and the paradoxical stimulatory effect at 10⁻⁷ M. GR79236 caused a dose dependent inhibition of noradrenaline and ADA stimulated lipolysis with a half-maximum effect at 11.3 ± 7.8 × 10⁻⁶ M (Fig. 1). To mimic the effect that an adenosine agonist may bring about in vivo, when endogenous adenosine would still be present, the experiments were repeated using noradrenaline alone for stimulation of lipolysis (Fig. 2). Under these circumstances GR79236 at a