Adenosine deaminase normalizes cyclic AMP responses of hypothyroid rat fat cells to forskolin, but not β-adrenergic agonists

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Lipolysis and cyclic AMP accumulation in response to β-adrenergic agonists or forskolin are severely impaired in fat cells from the hypothyroid rat. Incubating hypothyroid rat fat cells with adenosine deaminase normalizes the cyclic AMP response to forskolin, but not to β-adrenergic agonists. Increased sensitivity to adenosine action in the hypothyroid state appears to be the basis for the impaired cyclic AMP response to forskolin, but does not appear to be the underlying defect responsible for the impaired response to β-adrenergic agonists.

Hypothyroidism *Cyclic AMP* *Forskolin* *β-Adrenergic agonist* *Adenosine deaminase*

1. INTRODUCTION

Cyclic AMP accumulation and lipolysis of rat fat cells in response to β-adrenergic agonists is severely impaired in the hypothyroid state [1–4]. The hypothyroid rat fat cell provides a useful model for the study of the interactions between thyroid hormones and hormone-sensitive adenylate cyclase [5]. The number and affinity of β-adrenergic receptors of rat fat cells as measured by radiolabeled antagonist binding are unaffected by hypothyroidism [4,6]. Increased cyclic AMP phosphodiesterase activity [7], increased sensitivity to adenosine [8], and reduced signal transduction between the β-adrenergic receptor and the coupling protein (G/F[9] or N[10]) of adenylate cyclase [4] have each been suggested to be the basis for the insensitivity of hypothyroid rat fat cells to stimulation by β-adrenergic agonists.

The ability of the plant diterpen forskolin to stimulate cyclic AMP accumulation of rat fat cells was recently reported to be reduced by hypothyroidism [11]. Forskolin is a potent stimulator of cyclic AMP accumulation in intact cells [12] and activates adenylate cyclase in membranes devoid of functional G/F [13]. Here, we investigate the basis for the impaired response of hypothyroid rat fat cells to forskolin. Interestingly, adenosine deaminase was found to normalize the cyclic AMP response of hypothyroid rat fat cells to forskolin, but not to β-adrenergic agonists.

2. MATERIALS AND METHODS

Forskolin was purchased from Calbiochem (San Diego, CA), [2,8-^{3}H] cyclic AMP (34.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Crude collagenase from *Clostridium histolyticum* (130 units/mg, lot 4197CLS4ZC287) was purchased from Millipore (Freehold, NJ). Bovine serum albumin (Fraction V, lot 100F-0249), adenosine deaminase from calf intestinal mucosa (type III), (–)isoproterenol, and (–)epinephrine were purchased from Sigma (St. Louis, MO). Iodine-deficient test diet and the same diet to which iodine was added by the commercial supplier were purchased from United States Biochemical Corporation (Cleveland, OH). The en-

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zymes used in the glycerol assay were purchased from Boehringer (Indianapolis, IN). All other reagents were of the highest purity commercially available. Rats (150–175 g fed female Sprague-Dawley SD Strain) were purchased from Taconic Farms (Germantown, NY). Rats were rendered hypothyroid by maintenance on an iodine-deficient diet containing propylthiouracil for 14 days [4]. Fat cells were isolated from parametrial fat pads using collagenase treatment [14], and incubated in Krebs-Ringer phosphate buffer containing 3% bovine serum albumin and the indicated agents [4]. Forskolin was dissolved in 95% ethanol using a bath sonicator. The stock solution of forskolin was 10 mM and was diluted with water to obtain the concentrations indicated. Controls contained the same concentration of ethanol as the forskolin solutions.

3. RESULTS

Stimulation of lipolysis and cyclic AMP accumulation by epinephrine was reduced in fat cells from the hypothyroid rat (fig.1). Addition of adenosine deaminase (1 μg/ml) to the incubation media enhanced the lipolytic and cyclic AMP responses of both hypothyroid and euthyroid rat fat cells. Lipolysis and cyclic AMP accumulation of hypothyroid rat fat cells in response to epinephrine were not normalized, however, by the inclusion of adenosine deaminase. The amount of cyclic AMP accumulated by hypothyroid rat fat cells in the presence of adenosine deaminase (1 μg/ml) and epinephrine (10 μM) was only 20% of the amount accumulated by their euthyroid counterparts. Raising the concentration of adenosine deaminase in the incubation to 100 μg/ml, adding the adenosine-antagonist theophylline (1 mM), or combining theophylline (1 mM) with adenosine deaminase (1 μg/ml) also failed to rectify the blunted cyclic AMP response of hypothyroid rat fat cells to the stimulation of β-adrenergic agonists (not shown).

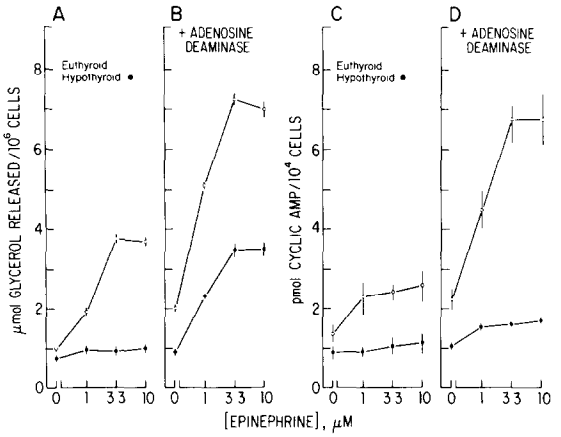

Fig.1. Effects of adenosine deaminase on the dose-dependent stimulation of glycerol release and cyclic AMP accumulation by epinephrine in fat cells from euthyroid and hypothyroid rats. Fat cells ($2.0 - 2.5 \times 10^4$ cells/ml) from euthyroid (○) and hypothyroid (●) rats were incubated with the indicated concentration of (-) epinephrine in the absence (panels A and C) or presence (panels B and D) of adenosine deaminase (1.0 μg/ml) at 37°C. Glycerol release was measured at 30 min and cyclic AMP accumulation was measured at 5 min following the addition of cells to the incubation vessels.

Forskolin (100 μM) stimulated a rapid accumulation of cyclic AMP in fat cells from hypothyroid and euthyroid rats (fig. 2). Half-maximal stimulation of cyclic AMP accumulation in response to 100 μM forskolin was achieved at 2–5 min and maximal stimulation within 10 min at 37°C. Cyclic AMP accumulation in response to 100 μM forskolin was found to be dramatically lower in fat cells obtained from hypothyroid as compared to euthyroid rats. Forskolin-stimulated cyclic AMP accumulation at 30 min of incubation was 2.45 ± 0.04 nmol/10^5 cells for euthyroid and only 0.28 ± 0.02 nmol/10^5 cells for hypothyroid rat fat cells. The p-value for the difference between these two values was less than 0.01 as evaluated by the Student's t-test.

In the presence of adenosine deaminase the time course for forskolin-stimulated cyclic AMP accumulation in fat cells from euthyroid and hypothyroid rats was also quite rapid (fig.2 right-hand panel). In sharp contrast to what was observed in cells incubated without adenosine deaminase, forskolin (100 μM) stimulated equivalent amounts of cyclic AMP accumulation in fat cells from hypothyroid and euthyroid rats in the presence of adenosine deaminase (1 μg/ml). Under these conditions, forskolin-stimulated cyclic AMP accumulation at 30 min was 17.3 nmol/10^5 cells in euthyroid and 19.8 nmol/10^5 cells in hypothyroid rat fat cells. This is the only condition identified in which the cyclic AMP response of hypothyroid rat

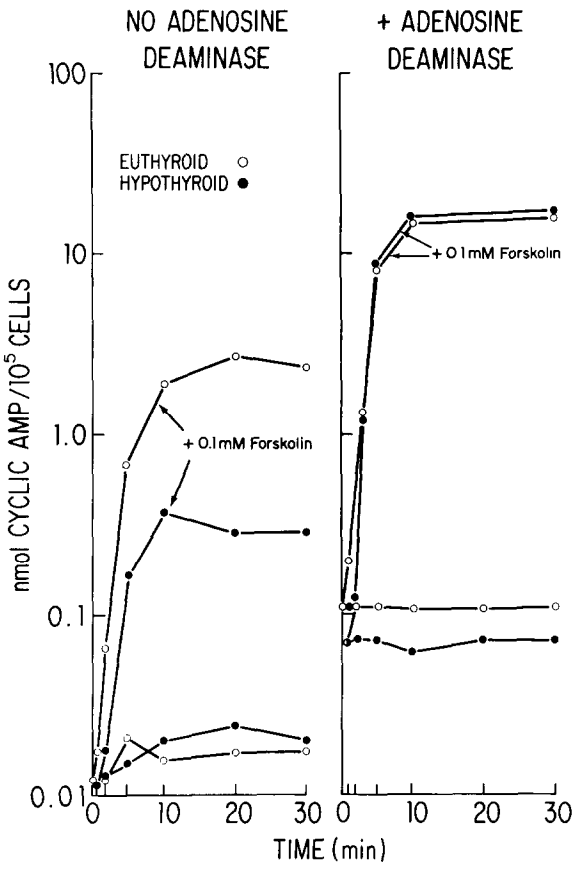

Fig.2. Time course for stimulation of cyclic AMP accumulation by forskolin in fat cells from euthyroid and hypothyroid rats. Fat cells ($4.0 - 4.5 \times 10^4$ cells/ml) from euthyroid (○) and hypothyroid (●) rats were incubated with and without 0.1 mM forskolin in the absence (left-hand panel) or presence (right-hand panel) of adenosine deaminase (1 μg/ml) at 37°C. Cyclic AMP accumulation was measured at the times indicated following the addition of the cells to the incubation vessel.

fat cells achieves parity with the response of fat cells from the euthyroid rat.

4. DISCUSSION

It has been reported [8] that adenosine deaminase normalized the lipolytic response of hypothyroid rat fat cells challenged with β-adrenergic agonists. These investigators however, compared the lipolytic response of hypothyroid rat fat cells incubated in the presence of adenosine deaminase to that of euthyroid rat fat cells incubated in the absence of adenosine deaminase [8]. Here, we show that adenosine deaminase enhances the lipolytic responses of both hypothyroid and euthyroid rat fat cells to β-adrenergic agonist stimulation and fails to normalize the blunted lipolytic and cyclic AMP responses observed in hypothyroidism.

Authors in [11] reported that forskolin was unable to stimulate cyclic AMP accumulation of fat cells from hypothyroid rats to the levels observed in fat cells from euthyroid rats. We too, observed an impaired cyclic AMP response to forskolin in the hypothyroid state and investigated the basis for this observation. In the presence of adenosine deaminase and a maximal concentration of forskolin, fat cells from euthyroid and hypothyroid rats accumulated equivalent amounts of cyclic AMP. These data support our earlier observations that fluoride- and cholera toxin-stimulated adenylate cyclase activities of fat cell membranes are not altered by hypothyroidism [4,15].

Adenosine deaminase degrades the adenosine released by fat cells, thereby eliminating the well-known inhibitory effect of this nucleoside on fat cell adenylate cyclase [16]. Fat cells from the hypothyroid rat accumulate and release normal amounts of adenosine [16]. Thus the inhibitory regulatory system of the rat fat cell adenylate cyclase appears to be normal or perhaps more sensitive in hypothyroidism, whereas the stimulatory regulatory component of the system appears to be markedly blunted. Increased sensitivity to adenosine may explain why fat cells from hypothyroid rats respond poorly to forskolin stimulation, but does not appear to be the major underlying defect responsible for the poor response of these cells to β-adrenergic agonist stimulation.

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