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Adenosine, Thyroid Status and Regulation of Lipolysis

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Adipocytes from hypothyroid rats do not respond to adrenaline with increased glycerol release. Adenosine deaminase largely restores lipolytic sensitivity. This effect is reversed by 2-deoxycoformycin, an inhibitor of the enzyme, and by N^6-(phenylisopropyl)adenosine, which is not deaminated. Lipolytic response of normal cells to adrenaline is only 50% inhibited by phenylisopropyladenosine, whereas in cells from hypothyroid rats blockade is total. Inhibition of 50% was seen at 100 and 1nM concentrations respectively. Insensitivity to adrenaline of hypothyroid-rat adipocytes can, at least partly, be explained by increased sensitivity to adenosine.

It has been repeatedly observed that fat-cells and adipose tissue obtained from hypothyroid rats are insensitive to adrenaline stimulation of lipolysis and cyclic AMP accumulation (Debons & Schwartz, 1961; Vaughan, 1967; Ichikawa *et al.*, 1971; Armstrong *et al.*, 1974; Correze *et al.*, 1974; Malbon *et al.*, 1978). Thyroid hormones have been reported to affect the number of β-adrenergic receptors in the heart (Ciaraldi & Marinetti, 1977; Williams *et al.*, 1977). However, there is now strong evidence from binding studies with dihydroalprenolol that the total amounts of β-adrenergic receptors in rat fat-cells (calculated per cell) are identical in the hypothyroid, normal and hyperthyroid states (Malbon *et al.*, 1978). Both increased phosphodiesterase activity (Armstrong *et al.*, 1974) and a defect in transduction of information between hormone receptors and adenylate cyclase (Malbon *et al.*, 1978) have also been suggested to be the cause of the catecholamine insensitivity of fat-cells prepared from hypothyroid rats. However, it is known that very high concentrations of adrenaline (100μM) can stimulate lipolysis, even in these cells (Malbon *et al.*, 1978).

The anti-lipolytic action of minute concentrations of adenosine is well documented (Dole, 1961; Raben & Matsuzaki, 1966; Ebert & Schwabe, 1973; Schwabe *et al.*, 1973, 1974; Fain, 1973; Fain *et al.*, 1972; Fain & Wieser, 1975). Isolated fat-cells excrete adenosine as the dephosphorylation product of adenine nucleotides. The amount of adenosine excreted is enough to inhibit lipolysis, sometimes even maximally (Schwabe *et al.*, 1973). This inhibitory action can also be overcome by high concentrations of adrenaline. This analogy between hypothyroid insensitivity to lipolytic agents and adenosine inhibition of adrenaline-stimulated lipolysis led us to consider the possibility that insensitivity to adrenaline of fat-cells from hypothyroid rats could be a result of increased sensitivity to adenosine.

Materials and Methods

Male Sprague–Dawley rats of average weight 125g were used. Hypothyroidism was induced chemically by feeding the rats on an iodine-free diet containing 0.15% of propylthiouracil (Remington diet TD-68221, Tekland Mills, Madison, WI, U.S.A.) for at least 20 days. This avoids possible complications of calcium metabolism which sometimes arise after surgical or radioiodide-induced thyroidectomy. Fat-cells were prepared by the method of Harwood *et al.* (1975). Incubations were done in plastic scintillation vials in 2ml of Krebs–Ringer/Tris buffer, pH 7.4, containing 125mM-Nacl, 5mM-KCl, 1mM-$CaCl_2$, 2.5mM-$MgCl_2$, 1mM-KH_2PO_4, 25mM-Tris/HCl and 2% bovine serum albumin. Detailed information is given in Table 1 and in the legends to the Figures. After the incubation, 200μl of 3M-$HClO_4$ was added, and the resulting precipitate was removed by centrifugation. A sample (1ml) of the supernatant was neutralized by KOH/KCl/imidazole as described by Lowry & Passonneau (1972). Glycerol was measured in these neutralized samples by an enzymic method described previously (Chernick, 1969).

Collagenase (type II from *Clostridium histolyticum*) and adenosine deaminase (EC 3.5.4.4; type I from calf intestinal mucosa; >200 units/mg of protein) were from Sigma, St. Louis, MO, U.S.A. The activity of the latter enzyme was checked by the method of Kalckar (1947). N^6-(Phenylisopropyl)adenosine was a gift from Dr. Harald Stork from Boehringer Mannheim G.m.b.H., Mannheim, W. Germany. The structure of this compound was checked by gas chromatography–mass spectrometry. 2-Deoxy-

coformycin (NSC 218321) was donated by Dr. John D. Douros from the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, Bethesda, MD, U.S.A.

Results and Discussion

First, we confirmed the finding that fat-cells prepared from rats made chemically hypothyroid do not readily respond to adrenaline by hydrolysis of intracellular triacylglycerol. If the cells were left for 15min before the start of the experiment, even very high concentrations (30 μM) of adrenaline did not stimulate glycerol release. However, when special care was taken in washing the cells immediately before starting the experiment, a relatively slight increase in glycerol release was seen at lower hormone concentrations (Fig. 1). This suggests that an inhibitory substance is excreted into the medium. This could well be adenosine (Schwabe *et al.*, 1973).

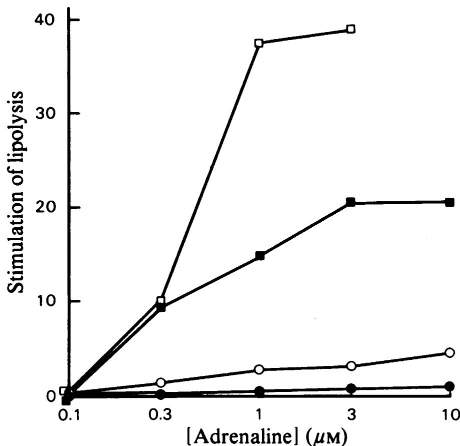

Fig. 1. *Effect of adenosine deaminase on the lipolytic sensitivity of hypothyroid-rat fat-cells*

Batches of 5×10^5 fat-cells were incubated in a volume of 2ml for 20min in the presence of different concentrations of adrenaline as shown. Adenosine deaminase was used at a concentration of 1 μg/ml.

Stimulation of lipolysis =

$$\left(\text{stimulated rate}\times\frac{1}{\text{basal rate}}\right)-1$$

The basal rates of glycerol release were 64 and 58 nmol/h per 10^6 cells for the normal and hypothyroid cell preparations respectively. ●, Hypothyroid; cells preincubated for 15 min; no adenosine deaminase. ○, Hypothyroid; no preincubation; no adenosine deaminase. ■, Hypothyroid; cells preincubated for 15 min; adenosine deaminase added. □, Normal; cells preincubated for 15 min; no adenosine deaminase added.

Table 1. *Inhibition of the lipolytic response to adrenaline and adenosine deaminase by N^6-(phenylisopropyl)adenosine and 2-deoxycoformycin in hypothyroid-rat adipocytes*

Batches of 3.8×10^5 cells were incubated in a total volume of 2ml in the presence of the compounds shown for 30 min as described in the Materials and Methods section. The unit of the rate of lipolysis is nmol of glycerol liberated/h per 10^6 cells. Results are means ± S.E.M. for five determinations.

Addition	Rate of lipolysis
None	46.3 ± 4.4
Adenosine deaminase (1 μg/ml)	56.2 ± 5.5
Adrenaline (0.5 μM)	62.7 ± 6.6
Adenosine deaminase + adrenaline	310.0 ± 21.0
Adenosine deaminase + adrenaline + deoxycoformycin (10 nM)	51.3 ± 5.5
Adenosine deaminase + adrenaline + N^6-(phenylisopropyl)adenosine (0.5 μM)	65.0 ± 6.3

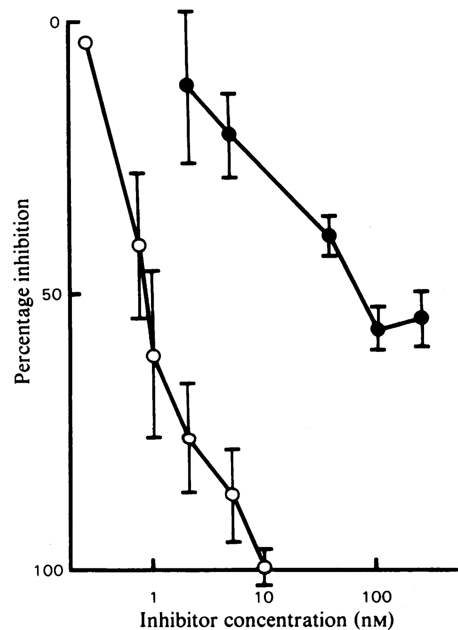

Fig. 2. *Inhibition of adrenaline stimulation of lipolysis by N^6-(phenylisopropyl)adenosine in fat-cells from normal and hypothyroid rats*

The cells were incubated for 30 min in the presence and absence of adenosine deaminase (1 μg/ml), adrenaline (0.5 μM) and different concentrations of N^6-(phenylisopropyl)adenosine. The basal and stimulated rates of glycerol release were 44.4 and 283 nmol/h per 10^6 cells for the normal and 26.4 and 172 nmol/h per 10^6 cells for the hypothyroid-rat fat-cells respectively. Vertical bars indicate the S.E.M. for five determinations. ○, Hypothyroid, 1.2×10^5 cells/ml; ●, normal, 1.9×10^5 cells/ml.

Adenosine deaminase was accordingly added to the medium. In the presence of this enzyme, 0.3μM-adrenaline was lipolytic (Fig. 1). In several replications of these experiments, 0.3μM-adrenaline was lipolytic, 0.1μM had a marginal effect and 0.075μM was ineffective. Thus the dose–response curve to adrenaline is shifted at least 300-fold to the left by adenosine deaminase.

In further experiments, the nature of the effect of the added enzyme preparation was investigated. The lipolytic response of the cells prepared from hypothyroid animals to 0.5μM-adrenaline in combination with adenosine deaminase was totally inhibited by addition of 0.5μM-N^6-(phenylisopropyl)adenosine, a derivative of adenosine that is not a substrate for adenosine deaminase (Westermann *et al.*, 1969) (Table 1). The lipolytic response was also abolished by 2-deoxycoformycin, an inhibitor of adenosine deaminase (Sawa *et al.*, 1967; Fain & Wieser, 1975). Hence it seems clear that the effect of the enzyme preparation is due to the specific action of the enzyme adenosine deaminase.

It is known that, in normal cells, adenosine and N^6-(phenylisopropyl)adenosine can only inhibit the lipolytic response to adrenaline by about 50% (Ebert & Schwabe, 1973). The data in Fig. 1 and Table 1 suggest increased sensitivity to these compounds in fat-cells prepared from hypothyroid rats. To confirm this, the inhibitory effects of different concentrations of phenylisopropyladenosine in normal adipocytes and adipocytes from hypothyroid rats were compared (Fig. 2). In normal cells, the dose–response curve was essentially identical with that reported by Ebert & Schwabe (1973), with about 50% maximal inhibition, which was observed at about 100nM concentration. In contrast, 5nM-N^6-(phenylisopropyl)adenosine prevented the increase in glycerol release totally in adipocytes prepared from hypothyroid rats. In several experiments, half-maximal inhibition was seen at 1–3nM concentrations, which clearly indicates increased sensitivity to this adenosine analogue.

While this manuscript was in preparation, Fernandez & Saggerson (1978) reported that adonosine deaminase can correct the altered sensitivity to insulin of fat-cells prepared from adrenalectomized rats. They propose that changes in adipocyte hormone responsiveness after adrenalectomy may result from changes in adenosine metabolism or release. It is possible that adenosine plays a more general role in the modulation of hormonal responses by other hormones or by the metabolic status of the cell itself.

Although in this communication we show that inhibition of the response to adrenaline is related to an increased sensitivity to adenosine in chemically induced hypothyroidism, it remains for further studies to determine whether fat-cells from hypothyroid rats might also secrete more adenosine than those from normal rats.

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