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Thyroid status and adenosine content of adipose tissue

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Fat-cells from hypothyroid rats lack the normal lipolytic response to catecholamines. We have suggested that this is due to increased sensitivity to the inhibitory actions of endogenous adenosine. In this paper we present evidence that thyroid hormones increase adipose-tissue adenosine contents and suggest that the increased sensitivity to adenosine in hypothyroidism is due to relief from desensitization by endogenous adenosine.

INTRODUCTION

Thyroid hormones play a permissive role in peripheral tissues, rendering them sensitive to the effects of catecholamines. It is well documented that fat-cells from hypothyroid rats lack the normal lipolytic response to physiological concentrations of catecholamines despite having a normal catecholamine-receptor status and a normal lipolytic machinery (Debons & Schwartz, 1961; Armstrong *et al.*, 1974; Malbon *et al.*, 1978). We have reported that this insensitivity *in vitro* is due to increased sensitivity to the inhibitory actions of endogenous adenosine (Ohisalo & Stouffer, 1979). This nucleoside has emerged as an important regulator of adipose-tissue metabolism. It can be formed from 5'-AMP by the action of nucleotides (Worku & Newby, 1983) and by hydrolysis of *S*-adenosylhomocysteine formed in methylation reactions from *S*-adenosylmethionine (Schrader *et al.*, 1981). It is also released together with the neurotransmitters from stimulated neurons as such or as a dephosphorylation product of nucleotides released from nerve granules (Fredholm & Hedquist, 1980). Adenosine has many insulin-like actions in adipose tissue. It inhibits cyclic AMP accumulation and lipolysis (Schwabe *et al.*, 1973; Ohisalo *et al.*, 1984; Kather *et al.*, 1985*a*,*b*) and stimulates lipoprotein lipase activity (Ohisalo *et al.*, 1981). The responsiveness of isolated fat-cells to adenosine has been reported to be increased in hypothyroidism (Ohisalo & Stouffer, 1979; Ohisalo, 1980; Chohan *et al.*, 1984; Malbon *et al.*, 1985; Saggerson, 1986; Woodward & Saggerson, 1986), though some investigators have not confirmed this finding (Fredholm & Vernet, 1984; Mills *et al.*, 1986). Changes in the response to adenosine have also been reported in isolated fat-cells after adrenalectomy (Saggerson, 1980), in obesity (Ohisalo *et al.*, 1986), during lactation (Vernon & Finley, 1985) and by aging (Hoffman *et al.*, 1984) and starvation (Chohan *et al.*, 1984).

Since the sensitivity to adenosine is increased in adipocytes from hypothyroid rats, it was considered important to find out if adenosine concentrations are altered as well.

MATERIALS AND METHODS

Male Wistar rats were kept under constant lighting conditions (06:00 h to 18:00 h). They were made hypothyroid by feeding them with a special low-iodine (0.5 p.p.m.) diet containing 0.15% propylthiouracil for 3 weeks. After this time the serum thyrotropin values of the rats on the test diet were 19.9 ng/ml on average (measured by radioimmunoassay versus NIAMDD r-TSH-RP2 standard) as compared with 1.3 ng/ml in control animals of the same age. In the beginning of the experiments the rats weighed about 150 g and, compared with control rats on a normal chow, their growth was insignificant. Because hypothyroid animals stop growing, it was not possible to compare hypothyroid and control rats of both same age and same weight. Further, the nutritional status of the hypothyroid rats may be quite different from that of control animals because of hormonal factors and possibly because the rats seem to dislike the test diet. Since both age (Hoffman *et al.*, 1984) and nutritional status (Chohan *et al.*, 1984) have been reported to affect the response of fat-cells to adenosine, comparing hypothyroid ainmals and animals on a normal diet might give misleading results. Therefore it was decided to compare hypothyroid rats on the test diet after injections of thyroid hormones or solvent. After 3 weeks on the test diet, the animals were given subcutaneous injections of thyroid hormones or solvent (150 mM-NaCl/5 mM-NaOH), as appropriate. The animals were anaesthetized with diethyl ether at the times indicated and the epididymal fat-pads were exposed surgically.

For studies of alterations of adenosine effectiveness, fat-cells were prepared by the method of Rodbell (1964), with the use of 0.5 mg of collagenase/ml of 4 mM-glucose/125 mM-NaCl/5 mM-KCl/2.5 mM-$MgCl_2$/1 mM-$CaCl_2$/1 mM-KH_2PO_4/25 mM-Tris/2% (w/v) bovine serum albumin at pH 7.4. After 60 min at 37 °C, the cells were washed three times with the same buffer without collagenase. The cells were then incubated in the presence of dialysed adenosine deaminase (100 munits/ml) or adenosine deaminase + isoproterenol (isoprenaline) and different concentrations of N^6-(phenylisopropyl)adenosine. After 60 min, the incubation was stopped by boiling. Glycerol was assayed luminometrically as described by Kather & Wieland (1984). In other experiments, 10–50 mg pieces were removed rapidly with tongs precooled in liquid N_2. The pieces were ground in liquid N_2 and their adenosine contents were assayed by radioimmunoassay as described elsewhere (Ranta *et al.*, 1985).

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Table 1. Effect of thyroid hormones on the adenosine content of adipose tissue in hypothyroid rats

Rats were rendered hypothyroid and injected with tri-iodothyronine (T_3) or thyroxine (T_4) (100 μg/100 g) in 150 mM-NaCl/5 mM-NaOH or with the solvent alone 24 h before being killed. In Expts. II and III, another similar injection of thyroxine was given 4 h before the rats were killed. Adenosine was assayed as described in the text. The adenosine values are given as means ± S.E.M.

	Injection	*n*	Adenosine (nmol/g of tissue)	Significance (two-tailed *t* test)
Expt. I	Solvent	4	0.31±0.16	$P < 0.05$
	T_3	8	0.85±0.14	
Expt. II	Solvent	3	0.32	$0.05 < P < 0.10$
	T_4	2	1.01	
Expt. III	Solvent	8	0.76±0.15	
	T_4	10	1.19±0.18	
Expts. I–III	Solvent	15	0.55±0.11	$P < 0.05$
	T_3 or T_4	20	0.87±0.09	

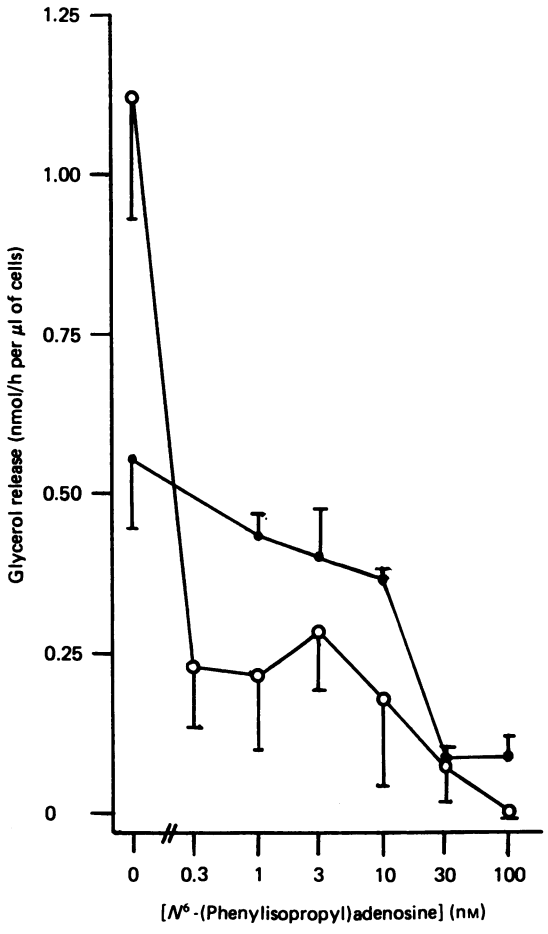

Fig. 1. Inhibition by N^6-(phenylisopropyl)adenosine of glycerol release from fat-cells from hypothyroid and thyroid-hormone-injected rats

Rats were made hypothyroid by giving them a low-iodine diet containing 0.15% propylthiouracil for 3 weeks. The animals were given subcutaneous injections of tri-iodothyronine (100 μg/100 g body wt.) or an equal volume of the solvent at 10:00 h on two consecutive days. The animals were then killed at 10:00 h on the third day, and fat-cells were prepared as described in the text. The cells were incubated for 60 min at 37 °C under constant shaking in the presence of adenosine deaminase (100 munits/ml) (tri-iodothyronine-injected animals) or adenosine deaminase + 0.15 μM-DL-isoproterenol (hypothyroid animals) and different concentrations of N^6-(phenylisopropyl)adenosine in a total volume of 400 μl. After the samples had been boiled for 1 min, glycerol was assayed chemilumimetrically. The points show means of four independent incubations. Vertical bars indicate S.E.M. ●, Cells from rats injected with tri-iodothyronine (+adenosine deaminase); ○, cells from rats injected with solvent (+adenosine deaminase and isoproterenol).

The low-iodine as well as the regular diets were prepared by Hankkija (Helsinki, Finland). Propylthiouracil was a gift from Eli Lilly. The antiserum against adenosine was a gift from Professor Andrew C. Newby (Cardiff, U.K.). N^6-(Phenylisopropyl)adenosine was a gift from Dr. Harald Stork. Collagenase (type II from *Clostridium histolyticum*) and adenosine deaminase (type I from calf intestine) were purchased from Sigma Chemical Co. Adenosine deaminase was dialysed against 150 mM-NaCl immediately before use.

RESULTS AND DISCUSSION

In comparing inhibition of lipolysis by adenosine analogues between cells from hypothryoid animals and such animals injected with tri-iodothyronine, it is important that endogenous adenosine is removed by adenosine deaminase and that both cell types have the same relative stimulus (Fredholm & Vernet, 1984; Saggerson, 1986). Preliminary experiments showed that lipolysis in cells from tri-iodothyronine-treated animals, in the presence of adenosine deaminase, was stimulated to about one-third of maximum even without the addition of isoproterenol. In cells from hypothyroid rats, maximal lipolysis was obtained in the presence of adenosine deaminase and 0.5 μM-DL-isoproterenol. Since we wanted to demonstrate that sensitivity to N^6-(phenylisopropyl)adenosine is decresed by tri-iodiothyronine injections, we chose these two conditions for comparison. The results are shown in Fig. 1 and indicate that cells from tri-iodothyronine-injected animals were less sen-

sitive to the adenosine analogue even though their lipolysis in the absence of the inhibitor was stimulated to a lesser degree in both relative and absolute terms.

The adenosine contents of adipose tissue in hypothyroid rats injected with thyroid hormones and the corresponding hypothyroid controls are shown in Table 1. It is obvious from this Table that thyroid hormones increased the adenosine content. The effect of triiodothyronine was statistically significant at $P < 0.05$ (Student's two-tailed t test), as was the difference between all control hypothyroid and all thyroid-hormone-treated animals ($n = 35$). One must remember that the values represent total amounts of adenosine per wet weight of tissue and that it is not known if these reflect the concentrations of free adenosine in the extracellular fluid in the tissue. The latter cannot be measured at present. It is known that part of adenosine is bound to intracellular proteins (Hershfield & Kredich, 1978). However, it is tempting to assume that the concentration of free extracellular adenosine would change in the same direction as total adenosine content. The cause of the decrease in adenosine content in hypothyroidism is unknown at present.

The present results confirm earlier findings that the sensitivity of fat-cells from hypothyroid rats to adenosine is increased (Ohisalo & Stouffer, 1979; Chohan *et al.*, 1984; Malbon & Graziano, 1983; Malbon *et al.*, 1985; Saggerson, 1986; Woodward & Saggerson, 1986). The molecular basis of this increased sensitivity is not known. Malbon *et al.* (1985) have suggested that it is due to changes in the relative amounts of the N-regulatory proteins in the plasma membrane. Whatever the mechanism, opposite changes in the concentrations of effectors and in the sensitivity of target cells to these effectors are often observed (for example, in the case of insulin). Indeed, it has been reported recently that chronic administration of N^6-(phenylisopropyl)adenosine, an adenosine R-site analogue, desensitizes rat fat-cells to the antilipolytic effect of the drug (Hoffman *et al.*, 1986; Parsons & Stiles, 1987). The present results show that the adenosine content of adipose tissue in the rat is increased by thyroid hormones. It is possible that the observed increase in sensitivity to adenosine in fat-cells from hypothyroid rats is due to relief from desensitization. Desensitization to hormones can often be partly explained by receptor down-regulation. However, it seems that in the case of desensitization to N^6-(phenylisopropyl)adenosine other factors are involved (Hoffman *et al.*, 1986). It has also been reported that adenosine receptor numbers are not increased in hypothyroidism (Chohan *et al.*, 1984; Malbon *et al.*, 1985), though there is very recent evidence that thyroid hormones can affect adenosine-receptor density in adipocyte plasma membranes (Rapiejko & Malbon, 1987).

Summing up, in the light of the present findings and previous literature, we suggest that adenosine concentrations in adipose tissue are decreased in hypothyroidism and that this leads to relief from desensitization of adipocytes to the actions of the nucleoside.

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