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MODULATION OF LIPOLYSIS BY ADENOSINE AND Ca^{2+} IN FAT CELLS FROM HYPOTHYROID RATS

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1. Introduction

Thyroid hormones are required to render peripheral tissues sensitive to β-adrenergic stimulation. Accordingly, catecholamines fail to stimulate lipolysis in adipocytes isolated from hypothyroid rats [1–6] whereas in normal fat cells the stimulatory effect is thoroughly documented. A decreased number of β-adrenergic receptors [2], disturbance in transduction of information between receptor and adenylyl cyclase [3], defective adenylyl cyclase [4] and increased phosphodiesterase activity [1,5] have all been suggested to be involved in this altered responsiveness.

Adenosine accumulates in preparations of isolated rat fat cells and it is an inhibitor of adenylyl cyclase activity [7]. Removal of this endogenous adenosine by added adenosine deaminase results in restoration of the lipolytic response to catecholamine stimulation [6]. The inhibitory effect of adenosine on adenylyl cyclase in broken fat cell preparations was reported dependent on the presence of Ca^{2+} and abolished by EGTA [8]. In [9] removal of Ca^{2+} from the incubation medium resulted in restoration of adrenaline-stimulated lipolysis to normal levels in fat cells prepared from hypothyroid rats.

This work was undertaken to investigate the possible involvement of external Ca^{2+} in the hypothyroid insensitivity towards catecholamine stimulation and the dependence on Ca^{2+} of the inhibitory actions of adenosine in fat cells.

2. Materials and methods

Male Sprague-Dawley rats (150 g initial wt) were used. The animals were made hypothyroid by feeding them an iodine-deficient diet containing 0.15% propylthiouracil (test diet TD 68221 from Teklad Mills, Madison, WI) for 3 weeks. Some of the animals received injections of T_3 (100 μg/kg body wt) on 3 consecutive days before killing. T_3 was dissolved in mild NaOH and the pH lowered to 7–8 by adding NaCl–HCl just before injection. The animals were guillotined and the epididymal fat pads removed. Fat cells were prepared by collagenase treatment [10] in a medium containing 125 mM NaCl, 5 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM KH_2PO_4, 25 mM Tris and 2% bovine serum albumin, at pH 7.4. The incubations were carried out in 2 ml in plastic scintillation vials using air as the gas phase. After 60 min, 200 μl 3 M $HClO_4$ was added and the samples were centrifuged at 3000 × g for 10 min. The supernatant fractions were neutralized by the method in [11]. Glycerol was determined by the α-glycerophosphate dehydrogenase/glycerokinase method [12].

Adenosine deaminase (200 units/mg protein, type 1 from calf intestinal mucosa), glycerokinase, α-glycerophosphate dehydrogenase, collagenase (type II from *Clostridium histolyticum*), L-isoproterenol–HCl, 3,3,'5-triiodo-L-thyronine (T$_3$) and fatty-acid free albumin were obtained from Sigma, St Louis, MO. The calcium ionophore A-23187 was from Eli Lilly, Indianapolis, IN). This compound was added as a solution in ethyl alcohol (ethel alcohol was final conc. 2 μl/ml which was also added in corresponding controls). N^6-(phenylisopropyl)adenosine was a kind gift from Dr Harald Stork.

3. Results

The results summarized in table 1 show that fat cells prepared from animals kept on the iodine-free

Table 1
Effect of thyroid hormones on the stimulation of lipolysis by isoproterenol

	Glycerol release ($nmol . min^{-1} . 10^6 cells^{-1}$)	
	Control	T_3
Control	0.82 ± 0.08	1.06 ± 0.06
Isoproterenol (1 μM)	1.07 ± 0.11	22.70 ± 4.48

The rats were kept on an iodine-free diet containing 0.15% propylthiouracil for 3 weeks. They received subcutaneous injections of T_3 (100 μg/kg body wt) or saline on 3 consecutive days before killing. The animals were killed 18 h after the last injection and the fat cells prepared as in the text. The cells were incubated at 10^5/ml for 60 min in the presence or absence of 1 μM isoproterenol as in the text. The values are means ± SEM of 5 determinations

diet containing propylthiouracil did not respond to 1 μM isoproterenol in so far as stimulation of glycerol release is considered. Subcutaneous injections of T_3 on 3 consecutive days to these animals before killing restored the lipolytic effect of the catecholamine. The buffer was the same as that used in the isolation of the cells.

To investigate the effects of external Ca^{2+}, fat cells were first prepared from hypothyroid rats as above. The cells were then washed with a buffer containing 127 mM NaCl, 5 mM KCl, 2.5 mM $MgCl_2$, 1 mM KH_2PO_4, 25 mM Tris and 2% bovine serum albumin, at pH 7.4. The washing was repeated 4 times. The washed cell preparation was divided into 2 parts, one of which was diluted 1:2 with the washing buffer in which 2 mM EGTA had been added with corresponding reduction in the concentration of NaCl. The other part was similarly diluted with the washing buffer containing 2 mM $CaCl_2$ and 123 mM NaCl. The adipocytes were then incubated for another 60 min as in section 2 at 2×10^5 cells/ml. The basal rate of lipolysis was somewhat lower in the presence of EGTA compared to the buffer containing calcium. Isoproterenol at 1 μmol/l failed to stimulate lipolysis in either group. However, in the presence of exogenous adenosine deaminase, lipolysis was strongly stimulated by the same concentration of isoproterenol in both systems. Again, lipolysis was slower in the presence of EGTA. Adenosine deaminase alone failed to stimulate lipolysis in these cells in repeated experiments. In another experiment, phenylisopropyladenosine was added. This compound is a potent inhibitor of adenylyl cyclase. It cannot be deaminated by adenosine deaminase because of its N^6-substitution. This compound at 5×10^{-7} M completely inhibited the stimulation of lipolysis. This effect was independent of the presence of Ca^{2+} (table 2). No essential differences from the above experiment were observed even if the cells were incubated for 60 min before the addition of isoproterenol. No significant stimulation of lipolysis by isoproterenol was observed in the calcium-free system without adenosine deaminase at 0.1 μM, 0.3 μM, 1 μM, 3 μM or 10 μM catecholamine. Neither was the regulation of lipolysis appreciably

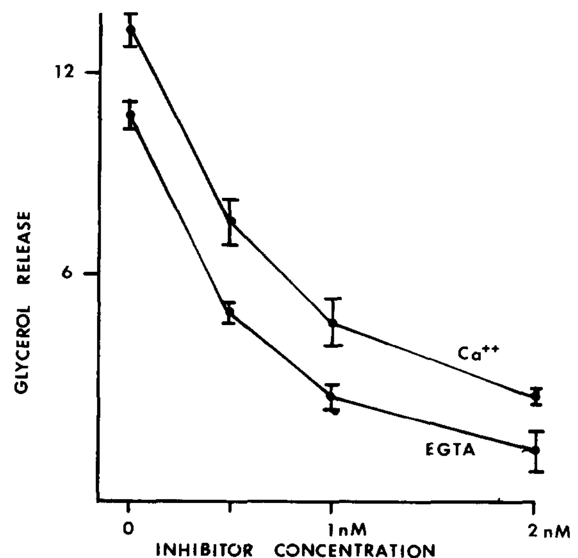

Fig.1. Inhibition of lipolysis by N^6-(phenylisopropyl)adenosine in the presence and absence of Ca^{2+}. Fat cells were prepared from hypothyroid animals and incubated at final conc. 3×10^5 cells/ml in the presence of 1 μM isoproterenol and 1 μg adenosine deaminase/ml. Glycerol release is expressed as $nmol . min^{-1} . 10^6 cells^{-1}$. Values are means ± SEM of 4 determinations.

Table 2
Effect of Ca^{2+} on the regulation of lipolysis in fat cells from hypothyroid rats

Addition	Glycerol release (nmol . min^{-1} . 10^6 $cells^{-1}$)	
	Ca^{2+}	EGTA
None	0.70 ± 0.17	0.59 ± 0.02
Adenosine deaminase (1 μg/ml)	0.92 (mean of two)	0.99 (mean of two)
Isoproterenol (1 μM)	0.98 ± 0.08	0.90 ± 0.17
Adenosine deaminase (1 μg/ml)+ isoproterenol (1 μM)	14.80 ± 2.42	7.80 ± 0.96
Adenosine deaminase (1 μg/ml)+ isoproterenol (1 μM)+ N^6-(phenylisopropyl)-Adenosine (0.5 μM)	0.98 ± 0.02	1.17 ± 0.38

Fat cells were prepared from hypothyroid rats as described in the text. The cells were washed with calcium-free medium and incubated in the presence of either 1 mM $CaCl_2$ or 1 mM EGTA (in the absence of Ca^{2+}) at final conc. 2 × 10^5 cells/ml for 60 min. The values shown are means ± SD of ≥5 determinations

affected in this system by omission of Mg^{2+} as well as Ca^{2+}. In another experiment, stimulation of lipolysis by isoproterenol and adenosine deaminase was studied in the presence of A-23817 and Ca^{2+} and also in buffer containing EGTA but no calcium. No significant difference was observed (table 3).

In further experiments, the dependence of adenosine inhibition of lipolysis on the presence of calcium in the medium was studied. As lipolysis could not be stimulated in rat cells prepared from hypothyroid rats without adenosine deaminase [6] and the concentration of adenosine in the medium would otherwise be unknown, we first removed endogenous adenosine by a deaminase preparation. Lipolysis was stimulated by 1 μM isoproterenol as above in both EGTA and Ca^{2+} buffers. Different concentrations of N^6-(phenylisopropyl)adenosine were then added. In both buffers, half-maximal inhibition was seen at ~0.5 nM inhibitor concentration. Here, again, both basal and stimulated rates of lipolysis were slightly higher in the presence of Ca^{2+}.

4. Discussion

These results confirm our finding that the hypothyroid insensitivity towards β-adrenergic stimulation can be reversed by removing endogenous adenosine by adenosine deaminase [6]. Adenosine has been shown [13] to be involved in the modulation of the

Table 3
Stimulation of lipolysis in the presence of A-23187 in fat cells prepared from hypothyroid rats

Addition	Glycerol release (nmol . min^{-1} . 10^6 $cells^{-1}$)	
	EGTA	A-23187 + Ca^{2+}
None	1.7 ± 0.8	1.9 ± 0.8
Isoproterenol (1 μM)+ adenosine deaminase (1 μg/ml)	18.4 ± 0.3	15.7 ± 3.1

Fat cells were prepared and washed into a calcium-free buffer as described in the text. They were then incubated in the presence of either EGTA (1 mM) or Ca^{2+} (1 mM) and A-23187 (0.5 mM) for 1 h. The final cell conc. was 0.95 × 10^5/ml. The values are means ± SEM of 4 determinations

response of fat cells to catecholamines by glucocorticoids, which further suggests that adenosine is an important 'local hormone'.

The finding that omission of calcium sensitizes fat cells prepared from hypothyroid rats to catecholamines [9] could not be confirmed; rather, Ca^{2+} seemed to activate lipolysis in these cells. This is in accordance with reports on the role of Ca^{2+} in normal fat cells [14,15]. Here, the inhibitory effect of adenosine was also found to be independent of the presence or absence of Ca^{2+}. While Ca^{2+} has been shown to be required for adenosine to exert its effect on adenylyl cyclase in broken fat cell preparations [8], no such effect has been observed using whole normal cells [14], either. Calcium is known to have profound effects on intracellular metabolism and it may be important in the events leading to stimulation of lipolysis by β-adrenergic stimulation. These results suggest, however, that extracellular calcium has little to do with the insensitivity towards adrenaline observed in hypothyroid fat cells.

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