Hypothyroidism abolishes the glycogenolytic effect of vasopressin, angiotensin II and A23187 but not that of α_1-adrenergic amines in rat hepatocytes

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1. INTRODUCTION

Thyroid hormones are known to affect the sensitivity of many tissues to a variety of hormones including adrenergic agents (review [1]). In liver cells from normal rats, the metabolic actions of epinephrine are mediated through α_1-adrenoceptors whereas in hepatocytes from hypothyroid rats both α_1- and β-adrenoceptors seem to be involved [2–5]. The increased β-adrenergic responsiveness seems to be due to an increased number of β-adrenoceptors [4]. The effects of thyroid status on hepatic α_1-adrenergic sensitivity are not as clear. Some authors [2,5] have found a marked decrease in α_1-adrenergic sensitivity in hepatocytes from hypothyroid rats, whereas others have found no change [3,4]. An imparied responsiveness to vasopressin and angiotensin II in hepatocytes from hypothyroid rats was reported in [6]. This finding is of special interest since these vasopressor peptides seem to share with α_1-adrenergic amines a cyclic AMP-independent calcium-signalling mechanism which is associated with phosphatidylinositol turnover [7,8]. Therefore, unless the loss of sensitivity to these peptides is due to a decreased number of receptors, a general loss of sensitivity for vasopressin, angiotensin II and α_1-adrenergic amines in the hypothyroid state would be expected.

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Here, the ability of epinephrine, vasopressin and angiotensin II to stimulate glycogenolysis and phosphatidylinositol (PI) labeling in hepatocytes from normal and hypothyroid rats was studied. The calcium ionophore A23187 was employed to bypass the transduction mechanisms for calcium-signalling.

Our data show that hypothyroidism results in a marked insensitivity of rat hepatocytes to calcium; i.e., decreased sensitivity to vasopressin, angiotensin II and A23187. The α_1-adrenergic effect of epinephrine was not markedly affected, which suggests that other factors besides calcium are probably involved in α_1-adrenergic actions.

2. MATERIALS AND METHODS

6-*n*-Propyl 2-thiouracil, glucose oxidase, l-epinephrine, l-propranolol, arginine–vasopressin and angiotensin II were obtained from Sigma Chemicals. Collagenase and bovine serum albumin (fraction V) were obtained from Worthington and Reheis, respectively. Prazosin was a generous gift from Pfizer. $^{32}P_i$ (carrier free) was obtained from New England Nuclear.

Female Wistar rats (~200 g) were used. Control and experimental animals were littermates, of approximately the same weight, and both were fed ad libitum with purina rat chow. Hypothyroidism was induced by giving the rats water containing

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0.015% 6-*n*-propyl-2-thiouracil for 23–40 days. Hypothyroidism was assessed by decreased weight gain, dryness of fur and markedly decreased blood levels of triiodothyronine.

Hepatocytes were isolated between 8:00 and 9:00 a.m. by the Berry and Friend method [9] as modified [7]. Isolation, washing and incubation of the cells was done with Krebs–Ringer bicarbonate buffer, saturated with O_2/CO_2 (95%/5%) pH 7.4 at 37°C.

To determine the rate of glycogenolysis, cells were incubated for 60 min in the presence of the agents to be tested. The incubation was ended by cooling the cell suspensions in iced water. After 1 min centrifugation at top speed in a clinical centrifuge, the concentration of glucose in the supernatant was measured by the glucose oxidase procedure [10]. For the study of PI labeling, cells were incubated for 60 min in the presence of 10 μCi/ml $^{32}P_i$. Cell lipids were extracted with chloroform:methanol (2:1) and phospholipids were separated by one-dimensional thin-layer chromatography [11]. Radioactivity was counted in silica-gel scrappings of the phospholipids.

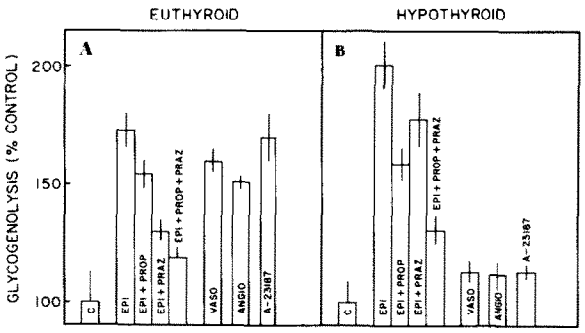

Fig. 1. Stimulation of glycogenolysis by vasopressin, angiotensin II, A23187 and adrenergic agents in hepatocytes from euthyroid (A) and hypothyroid (B) rats. Cells were incubated under the conditions in section 2 and in the presence of the agents indicated on each bar, at: 10^{-5} M epinephrine (EPI): 10^{-5} M propranolol (PROP); 10^{-5} M prazosin (PRAZ); 10^{-7} M vasopressin (VASO); 10^{-5} M angiotensin II (ANGIO); 10^{-5} M A23187. Bars are means (±SEM) of duplicate incubations from 3 cell preparations. Results are expressed as percentage of control (C) glucose output which was 67 ± 9 and 57 ± 5 nmol/mg cell wet wt for cells from euthyroid and hypothyroid rats, respectively.

3. RESULTS AND DISCUSSION

The stimulation of glycogenolysis by maximal concentrations of epinephrine, vasopressin angiotensin II and the ionophore A23187 in euthyroid and hypothyroid rats is presented in fig. 1. All the effects were dose-dependent; the dose–response curves have been ommitted for clarity. In euthyroid rats the effect of epinephrine was slightly diminished by the β-adrenergic antagonist propranolol, and was strongly diminished by the α_1-adrenergic antagonist prazosin. This indicates the predominant role of α_1-adrenoceptors in mediating the glycogenolytic response to epinephrine. Propranolol (10^{-5} M) and prazosin (10^{-5} M) were without effect on basal glycogenolysis (not shown). Vasopressin and angiotensin II, which seem to share the signal transduction system of α_1-adrenoceptors [7,8], strongly stimulated glucose output in hepatocytes from normal animals.

In cells from hypothyroid rats, the stimulation of glycogenolysis produced by epinephrine was stronger than in hepatocytes from normal animals. This stimulation was diminished to a similar extent by propranolol and by prazosin; the residual effect observed in the presence of either antagonist was significantly diminished when both antagonists

Table 1

Effect of vasopressin, angiotensin II and epinephrine on the incorporation of radioactive phosphate into phosphatidylinositol (PI) in hepatocytes from euthyroid and hypothyroid rats

Additions	Phosphatidylinositol labeling (% control)	
	Euthyroid	Hypothyroid
None	100	100
Epinephrine 10^{-5} M	291 ± 28	491 ± 28
Epinephrine 10^{-5} M + prazosin 10^{-5} M	115 ± 15	131 ± 16
Vasopressin 10^{-7} M	442 ± 29	502 ± 36
Angiotensin II 10^{-5} M	335 ± 33	279 ± 23

Cells were incubated under the conditions in section 2. Results are expressed as percentage of incorporation in the absence of hormones, and are the means (±SEM) of duplicate incubations from 3 cell preparations. Basal incorporation of label into phosphatidylinositol was 15 490 ± 3530 and 15 770 ± 3700 cpm/100 mg cell wet. wt in cells from euthyroid and hypothyroid rats, respectively

were present simultaneously. The adrenergic antagonists were without effect on basal glycogenolysis. This indicates that the glycogenolytic response to epinephrine in the hypothyroid state is a mixed α_1- and β-effect with strong contribution from both receptor types. The stimulation of glycogenolysis through activation of α_1-adrenoceptors (epinephrine + propranolol) was remarkably similar in cells from control animals ($154 \pm 6\%$ of control) to the stimulation produced in hepatocytes from hypothyroid rats ($159 \pm 7\%$ of control) (fig. 1). Surprisingly, in cells from hypothyroid rats, neither vasopressin nor angiotensin II were able to stimulate glycogenolysis (at any of the concentrations tested). Consistent with these findings we observed that vasopressin and angiotensin II did not stimulate gluconeogenesis or ureogenesis in hepatocytes from hypothyroid animals whereas α_1-adrenergic activation clearly stimulated these metabolic parameters in these cells (in preparation). A possible explanation for this hyposensitivity to the vasopressor peptides could be the absence of their respective receptors. To test this possibility, stimulation of PI turnover by these agents was studied. Dose–response curves for the stimulation of PI turnover by these agents were nearly identical in hepatocytes from normal and hypothyroid rats. The effects of maximal doses of the hormones of this parameter in hepatocytes from normal and hypothyroid rats is presented in table 1. This effect of epinephrine was blocked by prazosin (table 1). These data indicate that α_1-adrenoceptors, as well as the receptors for the vasopressor peptides, are present in the hypothyroid state, and are very probably coupled to their mechanism of signal transduction. Another possibility was that the sensibility of cells to the elevation in cytosol calcium brought about by these hormones could be altered in the hypothyroid state. The results obtained with ionophore A23187 are shown in fig. 1. The ionophore produced a strong glycogenolytic response in cells from euthyroid rats, whereas no response could be observed in cells from hypothyroid animals.

Although this insensitivity to calcium could explain the refractoriness of cells from hypothyroid rats to vasopressin and angiotensin II, it raises questions on the mechanism(s) involved in mediating α_1-adrenergic action. The accumulation of cyclic AMP due to epinephrine in cells from both control and hypothyroid rats was completely blocked by propranolol, indicating that cyclic AMP is not mediating the α_1-adrenergic effects in any of the thyroid states (not shown). Thus, a cAMP-independent calcium-independent mechanism is operating to bring about the α_1-adrenergic actions, which is readily observed in the hypothyroid state. The existence of at least two different transduction mechanisms for the α_1-adrenergic actions could also explain the different requirements for extracellular calcium between vasopressin, angiotensin II and α_1-adrenergic amines. The former two are strictly dependent on the presence of this ion whereas the latter is dependent to a much lesser extent.

The insensitivity to calcium brought about by hypothyroidism reveals that factor(s) besides calcium probably mediate α_1-adrenergic actions, and that such factors do not seem to be involved in the actions of the vasopressor peptides.

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