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EFFECT OF INSULIN ON ALPHA$_1$-ADRENERGIC ACTIONS IN HEPATOCYTES FROM EUTHYROID AND HYPOTHYROID RATS

Possible Involvement of Two Pathways in Alpha$_1$-Adrenergic Actions

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The effect of insulin on the alpha$_1$-adrenergic stimulation of glycogenolysis and ureogenesis, which is very small or undetectable in hepatocytes from control animals, is marked in hepatocytes from hypothyroid rats; the metabolic actions due to alpha$_1$-adrenergic activation, but not those due to glucagon, were nearly blocked by insulin in cells from hypothyroid rats. The alpha$_1$-adrenergic-mediated stimulation of phosphatidylinositol labelling was not affected by insulin in cells from either control or hypothyroid rats. The data suggest that the alpha$_1$-adrenergic action proceeds through two pathways, one of which is very sensitive to insulin and predominates in cells from hypothyroid rats.

The stimulations of glycogenolysis, gluconeogenesis and ureogenesis produced by epinephrine in liver cells from normal rats is an alpha$_1$-adrenoceptor-mediated, cyclic AMP-independent process (1-4). In common with the effects of vasopressin and angiotensin II, the action of alpha$_1$-adrenergic agonists is thought to be the result of changes in the calcium cytostasis (steady state concentration of Ca^{2+} in the different compartments of the cell) (reviewed in 5). Phosphatidylinositol (PI) turnover seems to be involved in the calcium signalling process due to activation of alpha$_1$-adrenoceptors or the receptors for the vasopressor peptides, vasopressin and angiotensin II (6-8). The role of PI and related phospholipids (phosphatidic acid, and phosphoinositides) in the calcium-signalling process has been a matter of dispute in recent years (9-13).

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It was recently shown that in hepatocytes from hypothyroid rats the glycogenolytic effect of vasopressin, angiotensin II or A23187 is decreased, but not that due to alpha$_1$-adrenergic activation (14). Interestingly, vasopressin, angiotensin II and epinephrine stimulate phosphatidylinositol labelling to a similar extent in cells from hypothyroid rats than in those from euthyroid rats (14). These data indicate that in cells from hypothyroid rats the action of vasopressin and angiotensin II is blocked at some step subsequent to receptor activation; these cells appear to be less sensitive to calcium-signalling (14). Consistent results have been obtained when metabolic pathways other than glycogenolysis were studied, i.e., no clear stimulation of gluconeogenesis or ureogenesis is produced by vasopressin or angiotensin II in hepatoyctes from hypothyroid female rats, whereas alpha$_1$-adrenergic activation clearly stimulates these metabolic processes in the same cells (15). All these data lead us to suggest that the action of alpha$_1$-adrenergic agonists may occur through two pathways, one of them (shared with vasopressin and angiotensin II) involving calcium as second messenger or coupling factor and another pathway, calcium-independent, which involves other unknown mediators (14-15).

Insulin is known to inhibit the sitmulation of glycogenolysis produced by glucagon and alpha$_1$-adrenergic agonists (16-21). In contrast, it has no effect on the stimulations of this pathway produced by vasopressin, angiotensin II or A23187(18-21).

In view of the above mentioned data, we hypothesize that the action of insulin could occur on the calcium-independent pathway involved in the alpha$_1$-adrenergic action. If so, the effect of insulin on the actions of alpha$_1$-adrenergic agents in hepatocytes from hypothyroid rats should be much greater than in hepatocytes from control animals. Our data confirm this possibility and support the involvement of two pathways in the alpha$_1$-adrenergic action.

MATERIALS AND METHODS

The sources of material were the same as in (4,14,15,22). Female Wistar rats (≈200 g) fed ad libitum were used. Hypothyroidism was induced by giving

the rats water containing 6-n-propyl-2-thiouracil for 23-40 days (14,15). Hypothyroidism was assessed by decreased weight gain, dryness of fur and decreased levels of triiodothyronine (14,15). Hepatocytes were isolated and incubated as described before (4,14,15,22). Glucose and urea were determined enzymatically (23,24). Cyclic AMP accumulation in the absence of theophylline was quantified after the addition of drugs by the method of Gilman (25). Phosphatidylinositol labelling was studied as described previously (14,15,22).

RESULTS

Epinephrine (in the presence of 10^{-5}M propranolol, to block its beta adrenergic activity) stimulated in a dose-dependent fashion the production of urea in cells from control or hypothyroid rats (Fig. 1) Insulin 10^{-9}M was without effect on the basal production of urea. We were unable to detect any clear cut effect of insulin on the stimulation of ureogenesis produced by epinephrine plus propranolol in hepatocytes from control animals. In contrast, insulin abolished the stimulation of ureogenesis produced by alpha$_1$-adrenergic activation of liver cells from hypothyroid rats (Fig. 1). Some stimulation of ureogenesis was produced in these cells by concentrations of epinephrine above

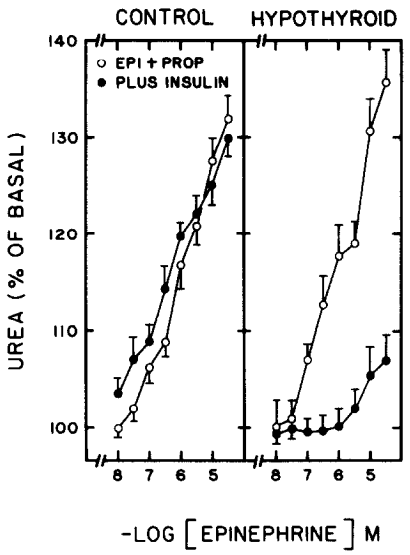

FIGURE 1.
EFFECT OF INSULIN ON ALPHA$_1$-ADRENERGIC STIMULATION OF UREOGENESIS IN HEPATOCYTES FROM CONTROL OR HYPOTHYROID RATS.

Cells were incubated for 60 min in buffer containing 1% albumin, 10 mM glucose, 10 mM glutamine, 2 mM ornithine, 10^{-5}M propranolol and different concentrations of epinephrine in the absence or presence of 10^{-9}M insulin. Results are expressed as percentage of basal values which were 19.5 $\pm$ 1.6 and 20.3 $\pm$ 1.5 nmol/mg cells for cells from control and hypothyroid rats; insulin was without effect on basal values. Plotted are the means and vertical lines represent the S.E.M. of 6 experiments in duplicate. EPI (Epinephrine), PROP (Propranolol).

10^{-6}M in the presence of insulin, but it could be due to incomplete beta adrenergic blockade by propranolol at these high concentration of epinephrine.

In order to determine if the action of insulin in cells from hypothyroid animals was unique for epinephrine or common to other agents, the effect of insulin on the ureogenic action of glucagon was studied. It was observed that insulin was unable to clearly antagonize the stimulation of ureogenesis produced by glucagon in cells from control or hypothyroid animals (Fig. 2)

The effect of insulin on the stimulation of glycogenolysis produced by submaximal concentrations of epinephrine plus propranolol or glucagon in cells from control and hypothyroid animals was studied. It was observed that insulin completely blocked the stimulation of glycogenolysis produced by epinephrine plus propranolol in cells from hypothyroid animals whereas it had nearly no effect on the alpha$_1$-adrenergic-mediated stimulation in liver cells from

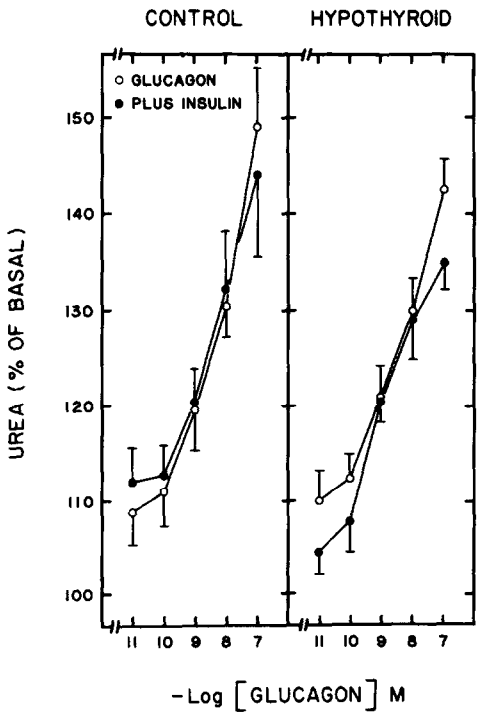

FIGURE 2.
EFFECT OF INSULIN ON THE STIMULATION OF UREOGENESIS IN HEPATOCYTES FROM CONTROL AND HYPOTHYROID RATS.
Cells were incubated as described in Fig. 1, in the absence of adrenergic agents but presence of different concentrations of glucagon. Other indications as in Fig. 1.

TABLE 1. EFFECT OF INSULIN ON THE STIMULATION OF GLYCOGENOLYSIS PRODUCED BY ALPHA$_1$-ADRENERGIC ACTIVATION OR GLUCAGON.

Additions	GLYCOGENOLYSIS (% OF BASAL)	
	Control	Hypothyroid
Basal	100	100
Insulin 10^{-9}M	114 ± 6	97 ± 3
Epinephrine 10^{-6}M		
+ Propranolol 10^{-5}M	178 ± 14	151 ± 8
Epinephrine 10^{-6}M		
+ Propranolol 10^{-5}M		
+ Insulin 10^{-9}M	152 ± 16	101 ± 5*
Glucagon 10^{-9}M	213 ± 9	165 ± 6
Glucagon 10^{-9}M		
+ Insulin 10^{-9}M.	188 ± 7**	171 ± 5

* $p < 0.001$ vs Epinephrine + propranolol (hypothyroid)

** $p < 0.05$ vs Glucagon alone (euthyroid)

Cells were incubated for 30 min in Krebs-Ringer bicarbonate buffer containing 1% albumin and the indicated agents. Basal glycogenolysis was 27.9 ± 2.6 and 32.9 ± 5.6 nmol/mg cells for control and hypothyroid rats respectively. Results are the means ± S.E.M. of duplicate determinations from 4 experiments.

control rats (Table I). The effect of 10^{-9}M glucagon was only slightly decreased by insulin in cells from either control or hypothyroid rats (Table I). The effect of insulin on the stimulation of PI labeling by epinephrine plus propranolol was also studied. It was observed that insulin did not modify this alpha$_1$-adrenergic action in cells from either control or hypothyroid rats (Fig. 3). It has been recently suggested that cyclic AMP may be involved in the action of alpha$_1$-adrenergic agonists (26). Therefore, the effect of insulin and epinephrine on this parameter were studied. Epinephrine alone produced 2-fold and 4-fold increases in the level of the cyclic nucleotide in cells from control and hypothyroid rats respectively (Table 2). However, it was observed that neither insulin, nor epinephrine plus propranolol produced any consistent effect on the levels of the cyclic nucleotide in cells from control or hypothyroid rats (Table 2).

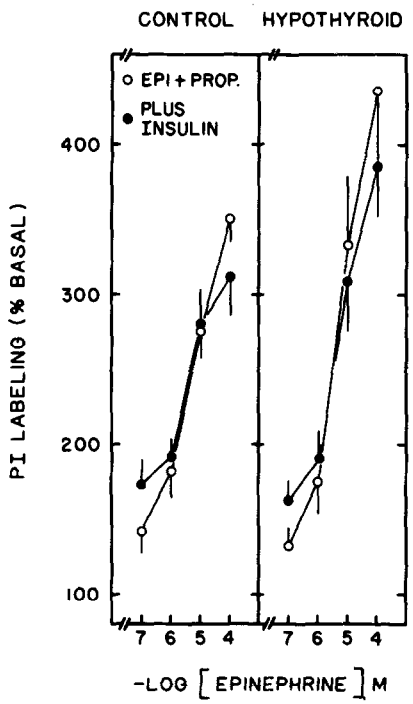

FIGURE 3.
EFFECT OF INSULIN ON THE ALPHA$_1$-ADRENERGIC STIMULATION OF PHOSPHATIDYLINOSITOL LABELING.

Cells were incubated under the conditions in Section 2. Results are expressed as percentage of the incorporation in the absence of hormones and are the means ± S.E.M. of duplicate incubations from 3 cell preparations. Basal incorporation of label was 6266 ± 695 and 4692 ± 1190 cpm/100 mg cells in cells from control and hypothyroid rats respectively. Other indications as in Fig. 1.

DISCUSSION

The present studies provide evidence that insulin markedly opposes the alpha$_1$-adrenergic-mediated stimulations of glycogenolysis and ureogenesis in hepatocytes from hypothyroid female rats. An increased responsiveness to insulin of liver cells from hypothyroid animals does not seem to explain the results because its effect specifically opposed the metabolic actions due to alpha$_1$-adrenoceptor activation.

Hepatocytes from control rats but depleted of calcium show remarkable similarities with hepatocytes from hypothyroid rats, i.e. it has been observed that in calcium-depleted hepatocytes the antagonism by insulin of glucagon action is not modified whereas insulin antagonism of phenylephrine action is markedly enhanced (18,19). Moreover, the actions of A23187 and vasopressin are abolished in these cells (18). However, there seem to exist differences

TABLE 2

EFFECT OF INSULIN, EPINEPHRINE AND EPINEPHRINE PLUS PROPRANOLOL ON CYCLIC AMP LEVELS.

Time min		Insulin 10^{-9}M	Epinephrine 10^{-6}M	Epinephrine 10^{-6}M + Propranolol 10^{-5}M	Epinephrine 10^{-6}M + Propranolol 10^{-5}M + Insulin 10^{-9}M
			Cyclic AMP (pmol/mg)		
	A.- Control				
0.5	0.53±0.04	0.58±0.04	----	0.60±0.04	0.61±0.04
1	0.52±0.05	0.48±0.06	1.12±0.12	0.45±0.04	0.50±0.06
5	0.50±0.06	0.52±0.02	----	0.43±0.04	0.40±0.02
10	0.39±0.03	0.50±0.03	----	0.38±0.06	0.36±0.02
20	0.39±0.03	0.43±0.03	----	0.36±0.04	0.37±0.05
	B.- Hypothyroid				
0.5	0.52±0.06	0.42±0.04	----	0.42±0.03	0.52±0.03
1	0.53±0.06	0.56±0.05	2.14±0.20	0.55±0.04	0.48±0.05
5	0.49±0.08	0.49±0.08	----	0.44±0.05	0.52±0.03
10	0.45±0.05	0.47±0.04	----	0.40±0.05	0.49±0.04
20	0.45±0.05	0.53±0.04	----	0.53±0.04	0.63±0.08

Cells were incubated in 1 ml of Krebs-Ringer bicarbonate containing 1% albumin and the indicated agents. Results are the means ± S.E.M. of 4 determinations.

between calcium-depleted hepatocytes from normal rats and non-depleted hepatocytes from hypothyroid rats, i.e., it has been observed that in calcium-depleted hepatocytes alpha$_1$-adrenergic activation stimulates cyclic AMP accumulation; it was therefore suggested that in such cells the effect of insulin opposing the alpha$_1$-adrenergic-mediated stimulation of glycogenolysis might be due to its ability to decrease cyclic AMP levels (18,19). There is no such alpha$_1$-adrenergic mediated accumulation of cyclic AMP in hepatocytes from hypothyroid rats. The mechanism through which alpha$_1$-adrenergic activation elevates cyclic AMP levels in calcium-depleted hepatocytes is obscure and the link between such increases in the level of the cyclic nucleotide and the metabolic actions remains to be established.

In hepatocytes containing normal amounts of calcium and obtained from normal animals, neither alpha$_1$-adrenergic agonists nor insulin alone or together altered the level of cyclic AMP (present paper and 19). However, insulin inhibits alpha$_1$-adrenergic effect under this condition (17-21).

It has been proposed that insulin may inhibit the binding of alpha$_1$-adrenergic agonists (20). Our data and those in (21) showing that insulin inhibits the metabolic effects produced by alpha$_1$-adrenergic amines but not their action on phosphatidylinositol labeling, strongly suggest that the action of insulin does not occur at the level of catecholamine binding to a homogeneous class of alpha$_1$-adrenoceptors.

Insulin inhibits the alpha$_1$-adrenergic-mediated release of calcium (19) but not the release of this cation induced by vasopressin and angiotensin II (19). Therefore it has been proposed that in normal cells insulin opposes alpha$_1$-adrenergic action by inhibiting calcium release (19). One possible explanation for these results is that the intracellular second messenger for the calcium-independent pathway of the alpha$_1$-adrenergic action could have secondary effects on calcium release and cyclic AMP levels, depending on the conditions of incubation. Insulin may regulate the level of the messenger or its actions.

The present data are circumstantial evidence supporting our hypothesis that the action of alpha$_1$-adrenergic agents involves two signals; a) one of them (shared with vasopressin and angiotensin II) which may involve PI turnover, calcium gating, elevation of cytosolic free calcium and seems to be insulin-insensitive and b) another pathway, calcium-independent cyclic AMP-independent and sensitive to insulin. Our data do not rule out the existence of different alpha$_1$-adrenoceptor subtypes associated to different transduction mechanisms. In summary our data indicate that the present schemes for hormone action are incomplete and furhter research is needed.

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