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Adrenergic regulation of gluconeogenesis: Possible involvement of two mechanisms of signal transduction in α_1-adrenergic action

(dihydroxyacetone/lactate)

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ABSTRACT **We have previously suggested that the effects of α_1-adrenergic agents on hepatocyte metabolism involve two mechanisms: (*i*) a calcium-independent insulin-sensitive process that is modulated by glucocorticoids and (*ii*) a calcium-dependent insulin-insensitive process that is modulated by thyroid hormones. We have studied the effect of epinephrine (plus propranolol) on gluconeogenesis from lactate and dihydroxyacetone. It was observed that the adrenergic stimulation of gluconeogenesis from lactate seemed to occur through both mechanisms, whereas when the substrate was dihydroxyacetone the action took place exclusively through the calcium-independent insulin-sensitive process. This effect was absent in hepatocytes from adrenalectomized rats, suggesting that it is modulated by glucocorticoids.**

It is well known that α_1-adrenergic agents, vasopressin, and angiotensin II stimulate glycogenolysis, gluconeogenesis, and ureogenesis in hepatocytes from normal rats through a cyclic AMP-independent mechanism associated with changes in the cytosolic concentration of calcium and with phosphoinositide turnover (1–4). Calcium, diacylglycerols, and inositol 1,4,5-trisphosphate are putative mediators of the action of these hormones (5–8).

During the last 4 years we (9–14) and others (15–22) have observed differences between the action of the vasopressor peptides and those due to α_1-adrenergic activation. These differences led us to propose the possible existence of two mechanisms of signal transduction for α_1-adrenergic action in the liver cell (9–14). Our hypothesis is schematically represented in Fig. 1 and is based mainly on the following findings: (*i*) metabolic effects due to α_1-adrenergic activation are clearly observed in cells incubated in the absence of extracellular calcium and even in calcium-depleted hepatocytes, whereas those of the vasopressor peptides are abolished (9, 14, 20); (*ii*) hypothyroidism markedly diminishes the metabolic effects of vasopressin and angiotensin II but not those due to α_1-adrenergic activation (11, 12); (*iii*) insulin reduces the stimulation of glycogenolysis due to α_1-adrenergic activation but not that produced by vasopressin or angiotensin II (13, 16, 17, 20); (*iv*) the inhibitory action of insulin on α_1-adrenergic actions is markedly magnified in calcium-depleted hepatocytes and in hepatocytes from hypothyroid rats (13, 16, 20); (*v*) in hepatocytes from adrenalectomized rats the metabolic effects due to α_1-adrenergic amines became dependent on the presence of extracellular calcium (14, 23)—i.e., α_1-adrenergic actions resemble those of vasopressin or angiotensin II; (*vi*) we have recently observed that cycloheximide, which stimulates hepatic metabolism through an α_1-adrenergic mechanism (24), mimics the actions of epinephrine in an insulin-insensitive calcium-dependent fashion and that the action of cycloheximide is observed in hepatocytes from control and adrenalectomized rats but not in cells from hypothyroid animals (25). Thus, in summary, our model suggests that α_1-adrenergic effects are mediated through two pathways: one of them also shared by vasopressin and angiotensin II, modulated by thyroid status, calcium-dependent, insulin-insensitive, and possibly involving phosphoinositide turnover and calcium in its mechanism of transduction; and another pathway, not shared with the vasopressor peptides, modulated by glucocorticoids, calcium-independent, insulin-sensitive, and mediated through unknown second messenger(s) (see Fig. 1).

Recently, Kneer and Lardy (15) reported that norepinephrine stimulates gluconeogenesis from dihydroxyacetone in the absence or presence of extracellular calcium. Interestingly, vasopressin and angiotensin II were unable to mimic the action of norepinephrine (15). These results prompted us to study comparatively the adrenergic regulation of gluconeogenesis from lactate and dihydroxyacetone in the light of our hypothesis, and the results are the subject of this manuscript.

MATERIALS AND METHODS

Materials. *l*-Epinephrine, *dl*-propranolol, glucose oxidase, peroxidase, arginine-vasopressin, angiotensin II, 6-*n*-propyl-2-thiouracil, lactate, dihydroxyacetone, cycloheximide, and EGTA, were obtained from Sigma. Bovine serum albumin (fraction V) and collagenase (type II) were obtained from Reheis (Kankakee, IL) and Worthington, respectively. Insulin was a generous gift from Eli Lilly.

Animals. Female Wistar rats (180–220 g) fasted 24 hr prior to the experiment were used. Hypothyroidism was induced by giving the rats water containing 0.030% 6-*n*-propyl-2-thiouracil for 30–40 days, and it was assessed by decreased weight gain, dryness of fur, and decreased blood levels of triiodothyronine (11, 12). Bilateral adrenalectomy was performed by a dorsal approach; adrenalectomized animals were given 0.85% NaCl to drink and were used 5–8 days after operation.

Hepatocyte Isolation and Metabolic Studies. Hepatocytes were isolated by the method of Berry and Friend (26) as modified by Tolbert *et al.* (2). Hepatocytes (≈40 mg, wet weight) were incubated for 60 min at 37°C in a water-bath shaker in 1 ml of Krebs–Ringer bicarbonate buffer containing 1% bovine serum albumin at pH 7.4 under an atmosphere of 95% O_2/5% CO_2.

In all the experiments, the cells were incubated in the presence of 1 μM propranolol to block the β-adrenergic activity of the agents studied. Propranolol by itself did not affect the parameters studied. Glucose was determined in aliquots of the supernatant by the glucose oxidase-peroxidase method (27). Glucose synthesis from exogenous substrates (10 mM lactate or 2.5 mM dihydroxyacetone) has been corrected for synthesis from endogenous metabolites by subtracting the glucose production in the absence of sub-

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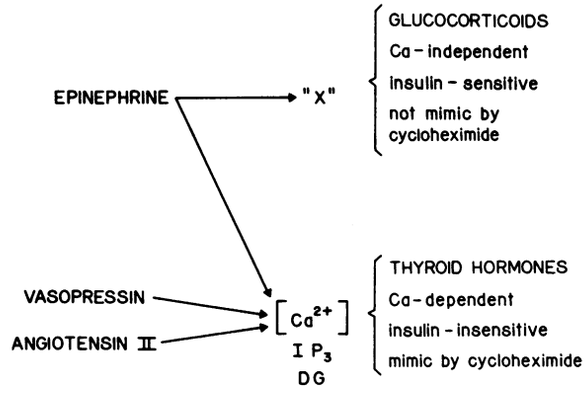

FIG. 1. Schematic representation of α_1-adrenergic action. IP_3, inositol 1,4,5-trisphosphate; DG, diacylglycerols.

strates from those values in the presence of substrate. All the data are the average of duplicate incubations of at least four different cell preparations.

RESULTS

Gluconeogenesis from Dihydroxyacetone and Lactate. Epinephrine, in the presence of 1 μM propranolol (to block its β-adrenergic activity) stimulated in a dose-dependent fashion the production of glucose from dihydroxyacetone or lactate (Fig. 2). The stimulation of glucose production from lactate was ≈60%, whereas that from dihydroxyacetone was only ≈20%.

To evaluate the role of extracellular calcium in the α_1-adrenergic-mediated stimulations of gluconeogenesis from these two substrates, cells were washed and incubated in buffer without $CaCl_2$ and containing 25 μM EGTA. Under these conditions, epinephrine was also clearly able to stimulate gluconeogenesis from both substrates (Fig. 2). Interestingly, the maximal stimulation of gluconeogenesis from dihydroxyacetone by epinephrine under these conditions was bigger (≈30%) than in the presence of calcium. In addition, the dose–response curve to epinephrine in the presence of lactate as substrate was shifted to the left (≈1 order of magnitude) in the absence of calcium as compared to the curve obtained in medium with calcium.

The effect of the vasopressor peptides, vasopressin and angiotensin II, on gluconeogenesis was studied and the results are presented in Fig. 3. In agreement with Kneer and Lardy (15), we observed that vasopressin and angiotensin II were ineffective in stimulating gluconeogenesis from dihydroxyacetone either in the absence or presence of extracellular calcium.

In contrast, both peptide hormones were able to stimulate gluconeogenesis from lactate in the presence of calcium; no effect of these peptides was observed in the absence of this cation (Fig. 3).

Studies with Hepatocytes from Hypothyroid Rats and Adrenalectomized Rats. Epinephrine in the presence of 1 μM propranolol stimulated gluconeogenesis from both dihydroxyacetone or lactate in hepatocytes from hypothyroid rats (Fig. 4). In contrast, in hepatocytes from adrenalectomized rats, epinephrine (plus 1 μM propranolol) was ineffective in stimulating gluconeogenesis from dihydroxyacetone but produced a clear dose-dependent stimulation of gluconeogenesis from lactate.

Effects of Insulin and Cycloheximide. The effect of insulin on the stimulations of gluconeogenesis from lactate or dihydroxyacetone by epinephrine is presented in Fig. 5. Insulin was without effect by itself. However, it abolished the stimulation of gluconeogenesis from dihydroxyacetone produced by epinephrine both in the presence or absence of calcium. In contrast, in the presence of lactate as substrate and in buffer containing calcium, insulin did not diminish the stimulation of gluconeogenesis produced by epinephrine. In the absence of calcium, insulin significantly diminished the stimulation of gluconeogenesis from lactate produced by epinephrine.

Cycloheximide, which seems to be a partial α_1-adrenergic

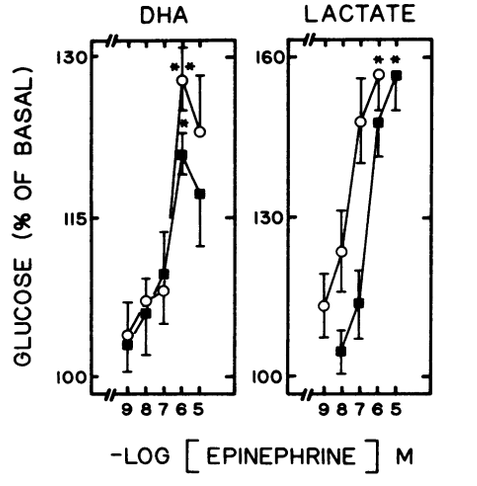

FIG. 2. Dose–response curves for the effect of epinephrine on gluconeogenesis from 2.5 mM dihydroxyacetone (DHA) or 10 mM lactate. Hepatocytes were incubated with substrates and agents for 60 min in the presence of 1.2 mM $CaCl_2$ (■) or in the absence of $CaCl_2$ and presence of 25 μM EGTA (○). Plotted are the means, and vertical lines represent the SEM of duplicate incubations from 4–8 cell preparations. Results are expressed as percentage of basal glucose synthesis from dihydroxyacetone, which was 18 ± 1 and 13 ± 1 nmol per mg of cells (wet weight) in the presence or absence of calcium, respectively, and from lactate, which was 7.9 ± 0.2 and 5.5 ± 0.4 nmol per mg of cells (wet weight) in the presence or absence of calcium, respectively, *, $P < 0.001$ vs. basal value; **, $P < 0.005$ vs. basal value.

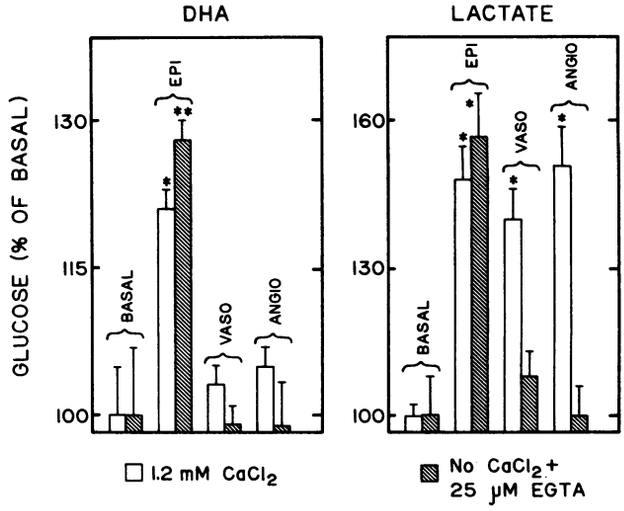

FIG. 3. Effect of 1 μM epinephrine and 1 μM propranolol (EPI), 1 milliunit of vasopressin (VASO), and 1 μM angiotensin II (ANGIO) on the stimulation of gluconeogenesis from 2.5 mM dihydroxyacetone (DHA) and 10 mM lactate in medium containing 1.2 mM $CaCl_2$ (open bars) or 25 μM EGTA without $CaCl_2$ (hatched bars). Incubation conditions were the same as those described in Fig. 2. Results are the means (±SEM) of duplicate incubations from 4–8 cell preparations and are expressed as percentage of basal glucose synthesis. Basal values are given in the legend to Fig. 2. *, $P < 0.001$ vs. basal values; **, $P < 0.005$ vs. basal value.

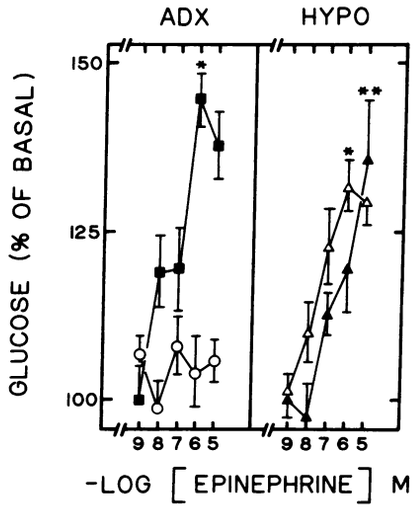

FIG. 4. Dose–response curves for the effect of epinephrine and 1 μM propranolol on gluconeogenesis from 2.5 mM dihydroxyacetone (open symbols) and lactate 10 mM (closed symbols). Hepatocytes isolated from adrenalectomized (ADX) or hypothyroid (HYPO) rats were incubated with substrates and agents for 60 min in the presence of 1.2 mM $CaCl_2$. Results are the means (±SEM) of duplicate incubations from 4–6 cell preparations and are expressed as the percentage of basal glucose synthesis, which was in cells from adrenalectomized rats, 16.2 ± 0.6 and 14.7 ± 0.9 from dihydroxyacetone and lactate, respectively, and in cells from hypothyroid rats, 14.9 ± 0.87 and 9.2 ± 0.85 nmol per mg of cell wet weight from dihydroxyacetone and lactate, respectively. *, $P < 0.001$ vs. basal value; **, $P < 0.02$ vs. basal value.

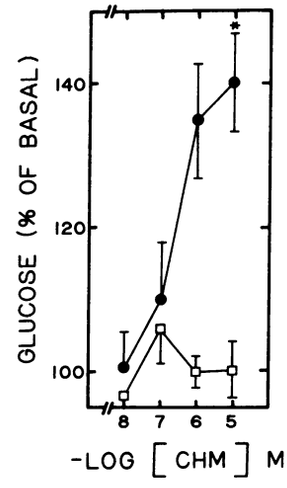

FIG. 6. Dose–response curve for effect of cycloheximide (CHM) on gluconeogenesis from 2.5 mM dihydroxyacetone (open squares) or 10 mM Lactate (closed circles). *, $P < 0.001$ vs. basal value.

agonist in liver cells (24, 25), stimulated in a dose-dependent fashion gluconeogenesis from lactate, but it was completely ineffective in doing so when dihydroxyacetone was the substrate (Fig. 6).

DISCUSSION

Several lines of evidence have suggested that some differences in the action of vasopressin and angiotensin II may exist, and we have proposed for the hypothesis that α_1-adrenergic action involves two mechanisms—i.e., the "conventional" mechanism shared with vasopressin and angiotensin II and an "alternative" mechanism (see Fig. 1) (9–14). Gluconeogenesis from lactate seems to be modulated by both mechanisms. In contrast, synthesis of glucose from dihydroxyacetone seems to be exclusively modulated by one of the pathways of the α_1-adrenergic action—i.e., the alternative pathway. Several criteria were fulfilled for this conclusion: (*i*) the effect of epinephrine is not mimicked by vasopressin, angiotensin II, or cycloheximide (Figs. 3 and 6); (*ii*) this action of epinephrine is not dependent on the presence of extracellular calcium (in fact, the effect is even bigger in the absence of extracellular calcium; Fig. 2); (*iii*) it is very sensitive to the action of insulin (Fig. 5); and (*iv*) it can be observed in hepatocytes from hypothyroid rats but not in cells from adrenalectomized animals (Fig. 4).

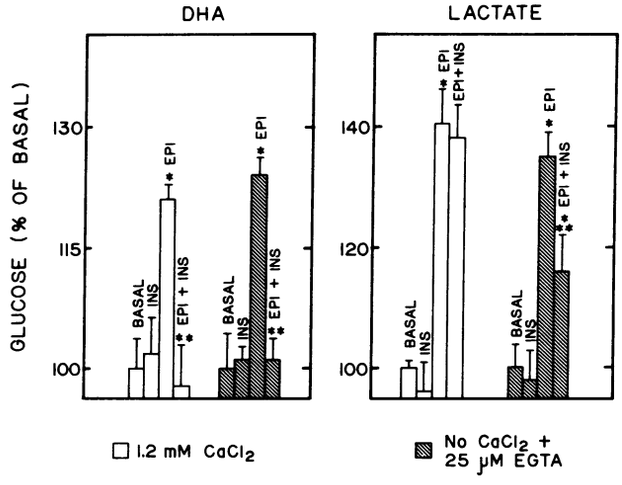

FIG. 5. Effect of insulin on stimulation of gluconeogenesis from 2.5 mM dihydroxyacetone (DHA) and 10 mM lactate. Hepatocytes from control rats were incubated in medium containing 1.2 mM $CaCl_2$ (open bars) or 2.5 μM EGTA without $CaCl_2$ (hatched bars) in the presence of 1 μM epinephrine and 1 μM propranolol (EPI), 1 μM epinephrine/1 μM propranolol/0.1 μM insulin (EPI + INS) or 0.1 μM insulin (INS). *, $P < 0.001$ vs. basal value; **, $P < 0.001$ vs. EPI; ***, $P < 0.02$ vs. EPI.

Our results are in close agreement with those of Kneer and Lardy (15). However, there is a difference in our findings; these authors did not observe an effect of adrenergic agents on gluconeogenesis from lactate in the absence of calcium (15). The reason for this is unclear at present. These authors used norepinephrine rather than epinephrine, and only at one concentration.

The effect of epinephrine on gluconeogenesis from lactate seems to be mediated by both pathways of α_1-adrenergic action but, interestingly, the dose–response curve to the agonist is shifted to the left in the absence of calcium as compared to the control (Fig. 2). This is surprising because actually we expected the opposite to occur, and it suggests that some amplification of the α_1-adrenergic action may take place under this condition. The effects of α_1-adrenergic agents are thought to occur through mechanisms independent of cyclic AMP (1–8). However, when the cells are incubated in the absence of calcium, the situation is somewhat more complicated. Under this condition, α_1-adrenergic activation reportedly increases cyclic AMP levels (23, 28, 29). Furthermore, it has been suggested that α_1-adrenoceptors become simultaneously coupled to two signal transduction mechanisms: calcium mobilization and cyclic AMP generation (19, 30). Therefore, a role of cyclic AMP in α_1-adrenergic action cannot be ruled out at the present. However, we have been unable to detect any significant stimulation of cyclic AMP formation by α_1-adrenergic activation, even in cells from hypothyroid rats where the alternative α_1-adrenergic pathway predominates (12). In addition, Lardy *et al.* (31) have observed that atractyloside inhibits the enhancement of

gluconeogenesis from dihydroxyacetone produced by cyclic AMP or glucagon but not the enhancement produced by epinephrine. These data raise some doubts on the metabolic significance of the reported α_1-adrenergic stimulations of cyclic AMP generation.

In summary, the data are consistent with our proposal of two mechanisms or pathways involved in α_1-adrenergic action. They also suggest that the α_1-adrenergic regulation of gluconeogenesis from dihydroxyacetone takes place through the pathway that is calcium-independent, insulin-sensitive, and modulated by glucocorticoids.

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