Adrenergic regulation of ureogenesis in hepatocytes from adrenalectomized rats

Possible involvement of two pathways of signal transduction in α_1-adrenergic action

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Received 22 October 1983

In hepatocytes from control rats, the ureogenic action of epinephrine is mainly mediated through α_1-adrenoceptors and the effect is independent of the presence of extracellular calcium. In hepatocytes from adrenalectomized rats, both α_1- and β-adrenoceptors are involved in the action of epinephrine. Furthermore, the α_1-adrenergic-mediated stimulation of ureogenesis in these cells is dependent on the presence of extracellular calcium. Our results indicate that glucocorticoids modulate the calcium dependency of α_1-adrenergic effects and are consistent with our suggestion that two pathways are involved in the transduction of the α_1-adrenergic signal.

α_1-Adrenergic receptor *Ureogenesis* *β-Adrenergic receptor* *Adrenalectomy*

1. INTRODUCTION

Thyroid hormones and glucocorticoids are known modulators of the actions of other hormones, including catecholamines (permissive effects). We have previously shown that in hepatocytes from hypothyroid rats the metabolic actions of vasopressin and angiotensin II are markedly diminished whereas the effects of α_1-adrenergic amines are not [1,2] but become extremely sensitive to the antagonistic action of insulin (submitted). The data suggested that the action of α_1-adrenergic agents may involve two pathways: one, shared with vasopressin and angiotensin II, which is dependent on extracellular calcium, probably involves phosphatidylinositol turnover and is insensitive to insulin; and the other, which is independent of extracellular calcium and sensitive to insulin [1,2]. We have therefore studied the effect of glucocorticoid deficiency (adrenalectomy) on hormonal regulation of ureogenesis. It was observed that in hepatocytes from adrenalectomized rats α_1-adrenergic stimulation of ureogenesis became dependent on the presence of extracellular calcium and was not affected by insulin.

2. MATERIALS AND METHODS

The sources of materials were the same as in [1–3]. Female Wistar rats (~200 g) fed ad libitum were used. 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. Hepatocytes were isolated and incubated under conditions to study

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

ureogenesis as in [1–3]. Urea was determined as in [4]. The dependency of extracellular calcium of hormonal effects was determined by adding EGTA (final concentration 2.5 mM, adjusted to pH 7.4) to the incubation buffer which contained 1.2 mM $CaCl_2$.

3. RESULTS

Epinephrine stimulated ureogenesis to the same extent in hepatocytes from control or adrenalectomized animals (fig.1). In contrast, isoproterenol was much more effective in hepatocytes from adrenalectomized animals than in control cells (fig.1) suggesting involvement of β-adrenoceptors in the action of epinephrine in cells from adrenalectomized animals. Studies with adrenergic antagonist were consistent with the findings, i.e., the effect of epinephrine was abolished by prazosin in hepatocytes from control animals, indicating that the action of the hormone is mediated mainly through α_1-adrenoceptors [3] (fig.2) whereas in hepatocytes from adrenalectomized rats only the addition of both prazosin and propranolol blocked

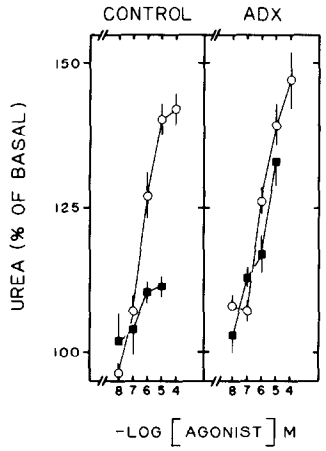

Fig.1. Effect of epinephrine or isoproterenol on ureogenesis in hepatocytes from control or adrenalectomized rats. Hepatocytes from control or adrenalectomized (ADX) rats were incubated in the presence of different concentrations of epinephrine (○) or isoproterenol (■). Means (± SE) are plotted of duplicate incubations from 4–8 cell preparations. Results are expressed as percentage of basal urea production during 60 min, which was 22 ± 2 and 25 ± 2 nmol/mg cell wet wt in cells from control and adrenalectomized rats, respectively.

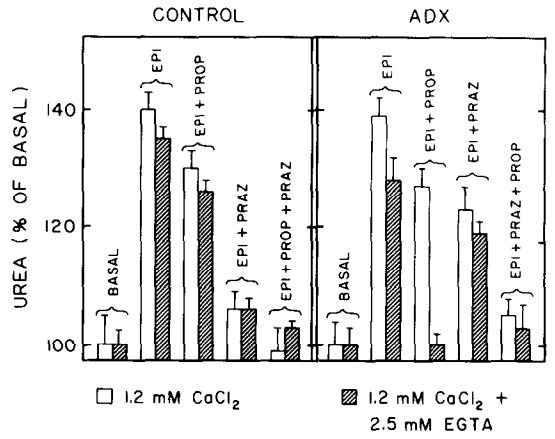

Fig.2. Effect of adrenergic antagonist on the stimulation of ureogenesis by epinephrine and role of extracellular calcium. Hepatocytes from control or adrenalectomized (ADX) rats were incubated for 60 min in medium containing 1.2 mM $CaCl_2$ (open bars) or 1.2 mM $CaCl_2$ plus 2.5 mM EGTA (hatched bars) in the presence of 10^{-5} M epinephrine (EPI); 10^{-5} M epinephrine + 10^{-5} M propranolol (EPI + PROP); 10^{-5} M epinephrine + 10^{-5} M prazosin (EPI + PRAZ) or 10^{-5} M epinephrine + 10^{-5} M propranolol + 10^{-5} M prazosin (EPI + PROP + PRAZ). Results are the means (± SE) of duplicate incubations from 4–8 cell preparations and are expressed as percentage of basal urea synthesis. Basal urea production in the presence of 1.2 mM $CaCl_2$ is given in fig.1; urea production in the presence of 1.2 mM $CaCl_2$ + 2.5 mM EGTA was 20 ± 1 and 29 ± 3 nmol/mg cells wet wt in cells from control and adrenalectomized rats, respectively.

the effect of epinephrine, showing that in these cells both α_1- and β-adrenoceptors are involved in the action of the amine (fig.2).

To evaluate further the α_1-adrenergic sensitivity of the cells, experiments were performed in the presence (1.2 mM $CaCl_2$) or absence (1.2 mM $CaCl_2$ plus 2.5 mM EGTA) of extracellular calcium (fig.3). In agreement with our previous studies [5], the ureogenic effect of epinephrine + propranolol is clearly observed in both the presence or absence of extracellular calcium in cells from control animals. Interestingly, in cells from adrenalectomized rats, the effect of epinephrine plus propranolol was abolished in the absence of calcium (fig.2,3). Some effect of epinephrine (in the presence of propranolol) was observed in cells incubated in the absence of calcium; however, it

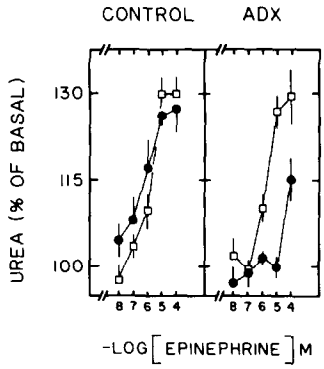

Fig.3. Role of extracellular calcium in the α_1-adrenergic-mediated stimulation of ureogenesis. Hepatocytes from control or adrenalectomized (ADX) rats were incubated for 60 min in the presence of 10^{-5} M propranolol and different concentrations of epinephrine in buffer containing 1.2 mM $CaCl_2$ (○) or 1.2 mM $CaCl_2$ + 2.5 mM EGTA (●). Results are the mean (± SE) of 4–8 cell preparations and are expressed as percentage of basal urea production. Basal rates of urea production are given in the legends to fig.1,2.

was observed at a very high concentration of epinephrine (10^{-4} M) at which incomplete β-adrenergic blockade by propranolol is very likely to occur. The absence of effect in medium containing EGTA was not due to general cell damage since clear effects of epinephrine alone or of epinephrine + prazosin were observed (fig.2). Vasopressin and angiotensin II stimulate ureogenesis in the presence of calcium in cells from either control or adrenalectomized rats (not shown).

Administration of dexamethasone to adrenalectomized rats (500 μg 48 and 24 h before the experiment was performed) restored the independence of extracellular calcium of the α_1-adrenergic stimulation of ureogenesis (116 ± 2% of basal level in the presence of 10^{-5} M epinephrine, 10^{-5} M propranolol and 2.5 mM EGTA; and 125 ± 2% of basal level in the presence of 10^{-4} M epinephrine + 10^{-5} M propranolol and 2.5 mM EGTA; means ± SE of 6 experiments). Furthermore, the effect of β-adrenergic agonists was significantly reduced in these cells as compared to that in cells from adrenalectomized rats not treated with the glucocorticoid.

We have previously shown that in cells from hypothyroid rats the α_1-adrenergic stimulation of ureogenesis is markedly diminished by insulin (submitted). In cells from adrenalectomized animals insulin was not able to antagonize significantly the effect of 10^{-5} M epinephrine plus 10^{-5} M propranolol (127 ± 3% of basal level in the absence of insulin as compared to 124 ± 2% in the presence of 10^{-9} M insulin; means ± SE of 8 experiments in each case).

4. DISCUSSION

Our findings here indicate that in hepatocytes from adrenalectomized rats β-adrenergic receptors play a significant role in the actions of epinephrine. The data are consistent with the observation by other authors that in liver from adrenalectomized animals the β-adrenergic-mediated activation of adenylate cyclase by epinephrine is enhanced [6,7]. This action seems to be related to an increased number of β-adrenoceptors in liver plasma membranes of adrenalectomized rats [8,9]. Our contribution in this respect is to show the involvement of β-adrenoceptors under this condition in a specific pathway: ureogenesis.

More relevant are our findings on α_1-adrenergic action. Previous studies have shown that the number of α_1-adrenoceptors in liver plasma membrane is not changed by adrenalectomy [10–12]. However, α_1-adrenergic effects are diminished in hepatocytes from adrenalectomized animals [10]. It has been observed that calcium depletion abolishes the effect of phenylephrine on phosphorylase in cells from adrenalectomized rats but not in control hepatocytes [10]. We confirmed this finding for ureogenesis and interpreted the data as suggesting that in hepatocytes from glucocorticoid-deficient rats epinephrine action proceeds mainly through the pathway shared with vasopressin and angiotensin II (dependency on extracellular calcium, insensitivity to insulin).

The effect of guanine nucleotides on the affinity state of α_1-adrenoceptors for agonists has recently been a matter of dispute [11–13]. It has been shown that such an effect of guanine nucleotides on α_1-adrenoceptors is modulated by glucocorticoids [13], i.e., the effect of guanine nucleotides is not observed in membranes from adrenalectomized rats but present in membranes from control animals and from adrenalectomized rats treated with glucocorticoids [13]. The

physiological role of guanine nucleotides (or a guanine nucleotide binding protein) in the process of signal transduction for α_1-adrenergic amines is far from clear. However, it is tempting to speculate that a relationship between the above-mentioned findings and our data may exist.

In summary, our data show that in hepatocytes from adrenalectomized rats the ureogenic effect of epinephrine is mediated by α_1- and β-adrenoceptors. Furthermore, in these cells the α_1-adrenergic action is dependent on the presence of extracellular calcium. Our data are consistent with the hypothesis that two pathways are involved in the α_1-adrenergic effects; one seems to be regulated by thyroid hormones whereas the other seems to be modulated by glucocorticoids.

ACKNOWLEDGEMENTS

This research was partially supported by a Grant from CONACyT (ICCBNAL 800637). The authors thank Ms Guadalupe Ramírez for typing the manuscript.

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