Angiotensin II inhibits the accumulation of cyclic AMP produced by glucagon but not its metabolic effects

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1. INTRODUCTION

The peptidic vasopressor hormones, vasopressin and angiotensin II, share the ability to stimulate hepatic glycogenolysis and gluconeogenesis [1–4]. These effects of the hormones are absolutely dependent on the presence of extracellular calcium [1–4]. It is generally accepted that both hormones have similar mechanisms of signal transduction which are cyclic AMP-independent and involve modulation of the cellular calcium fluxes [1–6]. Phosphatidylinositol turnover is associated with their effects and it might play a rôle in the process of signal transduction (reviewed in [7]).

Angiotensin II (but not vasopressin) inhibited adenylate cyclase in a purified preparation of liver plasma membrane [8]. The data suggest two receptors for angiotensin II in liver cells with different mechanisms of signal transduction: one of the receptors acting through a calcium-signalling process; the other through inhibition of adenylate cyclase.

The effect of angiotensin II and vasopressin on the actions of glucagon on cyclic AMP levels, glycogenolysis, gluconeogenesis and ureogenesis were investigated in hepatocytes incubated in calcium-free buffer.

2. MATERIALS AND METHODS

Angiotensin II, arginine-vasopressin, D,L-lactate (sodium salt), L-glutamine, L-ornithine, glycerol, glucose oxidase, peroxidase and urease were obtained from Sigma Chemical Co. Bovine serum albumin (fraction V) and collagenase (type II) were obtained from Armour and Worthington, respectively. Glucagon was a generous gift from Eli Lilly. [2,8–^{3}H]Adenosine 3′,5′-cyclic phosphate was obtained from New England Nuclear.

Hepatocytes were isolated from female Wistar rats as in [9]. The cells were washed and incubated in Krebs-Ringer bicarbonate buffer (without $CaCl_2$) containing 1% albumin and 0.5 mM EGTA (pH 7.4) under an atmosphere of O_2–CO_2 (95:5, v/v). Incubations were for 60 min at 37°C in 1 ml Krebs-Ringer bicarbonate buffer. Glucose production (in the absence of exogenous substrates) by hepatocytes from fed rats was considered as an index of glycogenolysis. Synthesis of glucose in the presence of 10 mM L-lactate by hepatocytes from 24 h fasted rats was considered as gluconeogenesis (glucose production in the absence of substrate represented 10–15% of the total and it was subtracted). Glucose was determined enzymatically [10]. Urea production was determined in cells from fed rats in medium supplemented with 2 mM ornithine and 10 mM glutamine and was determined as in [11]. Cyclic AMP accumulation was measured in cells from fed animals incubated in medium containing 100 μM theophylline, 2 min after the addition of the agents to be tested, as in [12]. Statistical significance of the difference between comparable groups was determined by Student's *t*-test.

3. RESULTS

Incubation of cells in calcium-free Krebs-Ringer bicarbonate buffer containing 0.5 mM EGTA

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resulted in a basal level of cyclic AMP of 0.36 ± 0.02 pmol/mg cells wet wt (mean ± SEM, n = 37). Vasopressin (10 mU/ml) and angiotensin II (10^{-6} M) were without significant effect on this basal value. Glucagon increased the level of the cyclic nucleotide in a dose-dependent manner reaching a maximum at 10^{-7} M (4.98 ± 0.33 pmol/mg cells wet wt; mean ± SEM, n = 39). Vasopressin (up to 10 mU/ml) was without effect on this glucagon-induced cyclic AMP accumulation (glucagon 10^{-7} M + vasopressin 10 mU/ml, 4.87 ± 0.40 pmol/mg cells wet wt; mean ± SEM, n = 16). However, angiotensin II produced a dose-dependent diminution of the cyclic AMP levels due to 10^{-7} M glucagon (fig.1). The maximal diminution (~ 50%) was obtained at 10^{-6} M angiotensin II (2.30 ± 0.23 pmol/mg cells wet wt, mean ± SEM, n = 39; $p < 0.001$ compared to glucagon alone).

The effect of the maximally effective concentration of angiotensin II (10^{-6} M) on the dose–response curves to glucagon for glycogenolysis,

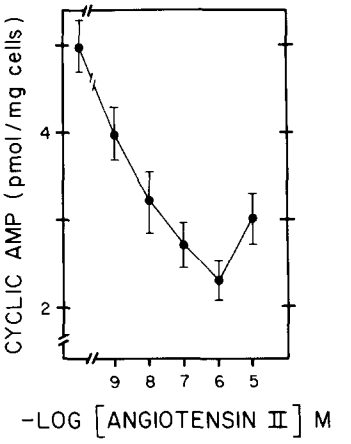

Fig.1. Dose–response curve for the effect of angiotensin II on the accumulation of cyclic AMP due to 10^{-7} M glucagon. Hepatocytes were incubated for 2 min in buffer containing 100 μM theophylline, 10^{-7} M glucagon and different concentrations of angiotensin II. Results are the means and vertical lines represent the SEM of 30–40 determinations.

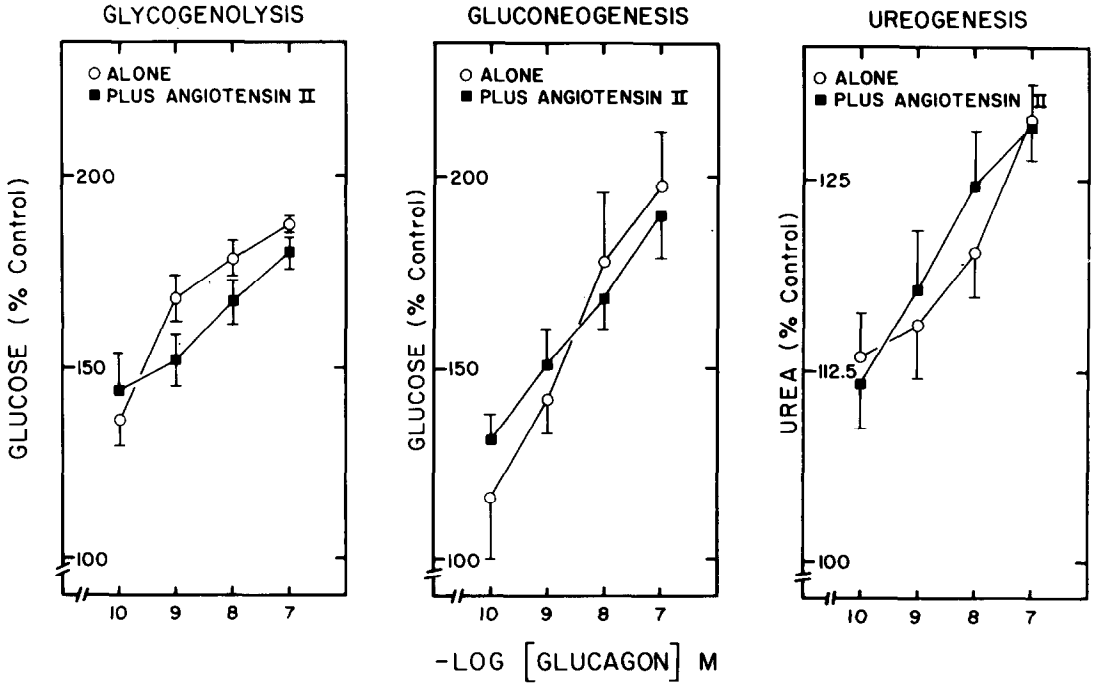

Fig.2. Dose–response curves for the effects of glucagon on glycogenolysis, gluconeogenesis and ureogenesis in the absence or presence of 10^{-6} M angiotensin II. Hepatocytes were incubated for 60 min with different concentrations of glucagon in the absence or presence of 10^{-6} M angiotensin II. Basal glycogenolysis was 21 ± 3 nmol/mg cells wet wt, basal gluconeogenesis 4.5 ± 0.2 nmol/mg cells wet wt and basal ureogenesis was 16.5 ± 1.5 nmol/mg cells wet wt. Results are the means and vertical lines represent the SEM of 4–8 cell prep.

gluconeogenesis and ureogenesis was studied. The dose–response curves in the absence or presence of angiotensin II were not significantly different (fig.2). Angiotensin II alone was without effect on these parameters (glycogenolysis 106 ± 3; gluconeogenesis 107 ± 5 and ureogenesis 107 ± 3; results are expressed as % of control value and are the means ± SEM of 4–8 cell preps).

4. DISCUSSION

These results confirm in whole hepatocytes the reported inhibition of adenylate cyclase by angiotensin II in liver plasma membranes [8], which suggests that the effect of the pressor hormone occurs in the membrane of parenchymal cells. In addition the effect of angiotensin II in whole cells is observed in calcium-free medium in contrast to its effects on glycogenolysis and gluconeogenesis [1–4]. Vasopressin did not share this ability to inhibit adenylate cyclase ([8], this work). Phosphatidylinositol labelling is another calcium-independent effect of angiotensin II. However, this effect and the calcium-dependent metabolic effects (stimulation of glycogenolysis and gluconeogenesis) are also produced by vasopressin. Our data support the suggestion [8] that two types of receptors for angiotensin II coupled to different mechanisms of signal transduction may exist in the liver cell. The possibility that a single type of receptor for angiotensin II (in contrast to the vasopressin receptor) might be coupled to both systems of signal transduction cannot be ruled out. However, a large amount of evidence suggests that some receptor subtypes are coupled to different systems of signal transduction (reviewed in [13,14]). The pharmacological characterization of receptor subtypes cannot be properly performed until selective agonists and antagonists become available.

The inhibition of adenylate cyclase by angiotensin II, as reflected by the decreased accumulation of cyclic AMP, was only observed when the cyclase was stimulated by glucagon. Monovalent cations stimulate adenylate cyclase in isolated membranes [8] and seem to be required to observe the inhibition of the cyclase by angiotensin II [8]. Both conditions (addition of monovalent ions in plasma membrane preparations and glucagon in whole cells) might represent better kinetic conditions, allowing observation of the inhibition.

It is generally accepted that the effects of glucagon are mediated through cyclic AMP: Angiotensin II which markedly decreases the accumulation of cyclic AMP due to glucagon should shift to the right the dose–response curves for glucagon. However, no shift or decrease in the maximal stimulation was detected. Therefore, this experiment provides a circumstance in which the effect of glucagon on cyclic AMP levels can be dissociated from its metabolic effects. Adenosine, which also inhibits adenylate cyclase, produces a similar dissociation [15]. Factor(s) besides cyclic AMP could be involved in glucagon action ([16], this work). Nevertheless, a pool of adenylate cyclase inaccessible to the receptors for angiotensin II might be responsible for the effects of glucagon.

Our results support the following suggestions:

(i) Two receptors for angiotensin II coupled to different mechanisms of signal transduction might exist in liver cells;

(ii) Factors besides cyclic AMP could play a role in the action of glucagon.

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REFERENCES

[1] Keppens, S. and De Wulf, H. (1975) FEBS Lett. 51, 29–32.

[2] Hems, D.A., Whitton, P.D. and Ma, G.Y. (1975) Biochim. Biophys. Acta 411, 155–164.

[3] Hems, D.A., Rodrigues, L.M. and Whitton, P.D. (1978) Biochem. J. 172, 311–317.

[4] Whitton, P.D., Rodrigues, L.M. and Hems, D.A. (1978) Biochem. J. 176, 893–898.

[5] Blackmore, P.F., Dehaye, J.P. and Exton, J.H. (1979) J. Biol. Chem. 254, 6945–6950.

[6] Strickland, W.G., Blackmore, P.F. and Exton, J.H. (1980) Diabetes 29, 617–622.

[7] Michell, R.H. and Kirk, C.J. (1981) Trends Pharmacol. Sci. 2, 86–89.

[8] Jard, S., Cantau, B. and Jakobs, K.H. (1981) J. Biol. Chem. 256, 2603–2606.

[9] Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506–520.

[10] Fales, F.W. (1963) Stan. Methods Clin. Chem. 4, 101–112.

[11] Gutman, I. and Bergmeyer, H.U. (1974) in:

Methods of Enzymatic Analysis (Bergmeyer, H.U. ed) pp. 1791–1794, Academic Press, New York.
[12] Gilman, A.G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[13] Fain, J.N. and García-Sáinz, J.A. (1980) Life Sci. 26, 1183–1194.
[14] Snyder, S.H. and Goodman, R.R. (1980) J. Neurochem. 35, 5–15.
[15] Fain, J.N. and Sheperd, R.E. (1977) J. Biol. Chem. 252, 8066–8070.
[16] Birnbaum, M.J. and Fain, J.N. (1977) J. Biol. Chem. 252, 528–535.