Stimulation of glycogenolysis in hepatocytes by angiotensin II may involve both calcium release and calcium influx

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The stimulation of [^{3}H]glucose release (a measure of glycogenolysis) from isolated guinea pig hepatocytes by Ca-mobilizing agonists can be resolved into two phases. The initial transient phase is independent of extracellular Ca, and is probably a result of Ca released from an intracellular pool. The second phase occurs only in the presence of extracellular Ca, which suggests that Ca-influx is also involved in the mechanism of Ca-mobilization by these agents in the guinea pig hepatocyte.

Ca^{2+} *Glucose-release* *Glycogenolysis* *Angiotensin II* *Ca^{2+}-influx* *Hormone receptor*

1. INTRODUCTION

Angiotensin II will activate liver glycogen phosphorylase by a mechanism that appears to be independent of cyclic AMP [1–4]. There is substantial evidence to indicate that ionized Ca is the second messenger for the glycogenolytic effect of angiotensin II. Liver phosphorylase *b* kinase is sensitive to Ca [5], and therefore its activity could be regulated by Ca, as has been shown for this enzyme in skeletal muscle [6]. Several investigators have found that extracellular calcium is important in the response to angiotensin II [2,4,7,8]. Authors in [9] proposed that angiotensin II (as well as vasopressin and α-agonists) caused an influx of calcium, which then resulted in activation of glycogen phosphorylase. They showed that in a medium without calcium plus 2 mM EGTA there was no activation of phosphorylase *a* by angiotensin II [2]. The Ca-ionophore A23187 also caused an activation of phosphorylase *a* that was greatly attenuated in the Ca-free medium. Angiotensin II stimulated the uptake of ^{45}Ca within 30 s after addition of the hormone. Authors in [7] confirmed the Ca-dependence of phosphorylase *a* stimulation by angiotensin II and showed that glucose release was even more critically dependent on the presence of extracellular Ca. They found that mere omission of Ca from the medium did not completely abolish the increase in phosphorylase *a* due to angiotensin II [7]; however, a more severe Ca-depletion (obtained by incubation in Ca-free medium plus 2 mM EGTA for 30 min) abolished this response [8].

Recently it has been shown that angiotensin II will cause a rapid net Ca efflux (measured with a Ca-selective electrode) from perfused rat livers [10]. The onset of this efflux precedes the increase in glycogenolysis, suggesting that the mobilization of an intracellular pool of Ca may be one of the earliest effects of angiotensin II, as has been proposed for the other cyclic AMP-independent agonists (review [11]).

We have demonstrated previously that another response to angiotensin II that appears to utilize Ca as a second messenger is the release of ^{86}Rb (a marker for K ions) from guinea pig liver [12,13].

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Abbreviation: EGTA, ethylene glycol bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid

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The stimulation of ^{86}Rb release by angiotensin II has an initial phase of release that is independent of the presence of extracellular Ca. In isolated guinea pig hepatocytes, there is a second phase of the response that requires extracellular Ca [13]. Here we report that the stimulation of glycogenolysis (measured as [^{3}H]glucose efflux) by angiotensin II in guinea pig hepatocytes also has both a Ca-independent and a Ca-dependent component. This suggests that the initial phase of the response, which is independent of extracellular Ca, is stimulated by Ca released from an intracellular pool. The second phase, which occurs only in the presence of extracellular Ca, is probably due to the influx of Ca into the cell. ATP (another agonist that is believed to activate phosphorylase by a Ca-dependent mechanism [14]) and the Ca-ionophore A23187 evoke very similar responses, thus indicating that both the release of intracellular Ca and the stimulation of Ca-influx are involved in the responses of liver cells to the cyclic AMP-independent agents.

2. MATERIALS AND METHODS

The preparation of isolated guinea pig hepatocytes has been described in [13]. The cells were pre-equilibrated for 1 h with 5–20 μCi/ml D-[6(*N*)-^{3}H]glucose in a GIBCO (Grand Island, NY) minimal essential medium supplemented with 2% bovine serum albumin (BSA) and containing 50 mM glucose to insure net glycogen synthesis and thereby to label the glycogen stores. The cells were then washed twice in the standard medium (which contained the same salt composition and 11 mM glucose) to remove the extracellular radioactivity. The standard medium contained (mM): NaCl, 116; KCl, 5.4; $CaCl_2$, 1.8; $MgSO_4$, 0.81; NaH_2PO_4, 1.0; $NaHCO_3$, 26; and D-glucose, 11.

The cells were drawn into perifusion-chambers (for details see [13]) and solution (without isotope present) was pumped through the system continuously at a rate of about 2 ml/min. To measure the unidirectional efflux of [^{3}H]glucose from the cells, fractions of the effluent were collected at 1-min intervals in scintillation vials, scintillation cocktail was added, and the samples were counted in a Beckman LS100C scintillation counter. At the end of an experiment, 5% Triton X-100 was pumped through the system to release the radioactivity remaining in the cells. The cellular samples were also collected and counted, enabling the calculation of apparent first-order rate coefficients (expressed in units of %/min). The radioactivity released during perifusion, in the presence or absence of hormone, was found to be >90% [^{3}H]glucose, as determined by paper chromatography.

Angiotensin II (Human) and its antagonist sar^{1}ile^8-angiotensin II were obtained from Peninsula Labs (San Carlos CA). ATP was from Sigma (St Louis MO). A23187 came from Calbiochem (La Jolla CA). D-[6(*N*)-^{3}H]glucose and scintillation cocktail were purchased from New England Nuclear (Boston MA). Other materials are referred to in [13].

3. RESULTS AND DISCUSSION

The addition of 10^{-7} M angiotensin II to the standard perifusion medium (1.8 mM Ca) evokes a biphasic increase in the release of [^{3}H]glucose from guinea pig hepatocytes whose glycogen stores have been pre-labelled with the isotope (fig.1). There is a large initial increase in the [^{3}H]glucose release which is followed by a second, slowly falling phase of the response.

This response can be resolved into two components; an initial transient phase which is independent of extracellular Ca, and a second phase which is dependent on the presence of Ca in the medium (fig.2). Perifusion of the cells with 10^{-7} M angiotensin II in a medium containing no added Ca and 0.1 mM EGTA (hereafter referred to as the zero-Ca medium) elicits a transient increase in [^{3}H]glucose release that lasts about 10 min. If the medium is changed to one that contains 3 mM Ca and 10^{-7} M angiotensin II, the second phase of the response is restored. The presence of 10^{-7} M sar^{1}ile^8-angiotensin II (an angiotensin II antagonist) from 15–50 min blocks both phases of the response, suggesting that the response is receptor-mediated. The change from a medium without Ca to one containing Ca does not alter the rate of [^{3}H]glucose release unless the agonist is present, therefore the second response is not due to the addition of Ca to a Ca-deficient medium.

ATP is another agonist that has been shown to activate phosphorylase *a* in the liver [14],

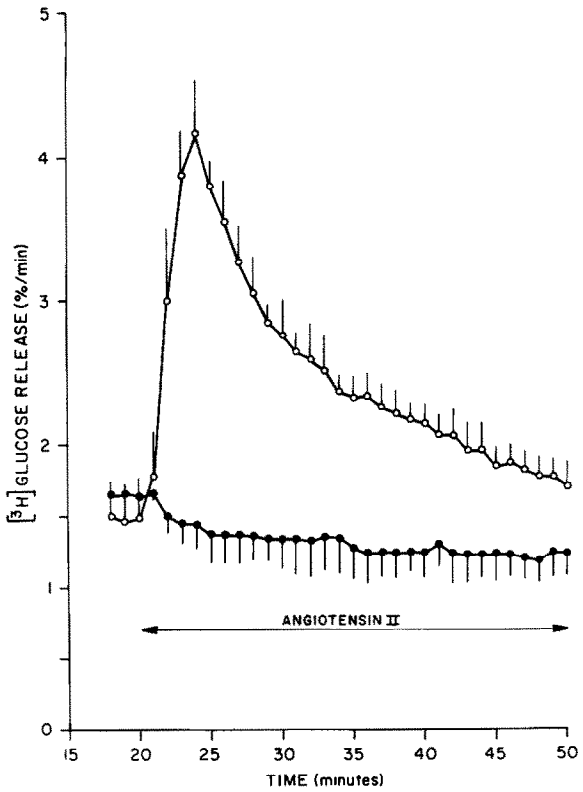

Fig.1. Effect of angiotensin II on [^{3}H]glucose release with 1.8 mM Ca present in the perifusion medium. Cells perifused with 10^{-7} M angiotensin II from 20–50 min (○), or without the agonist (●). Means and SEMs from 3 experiments.

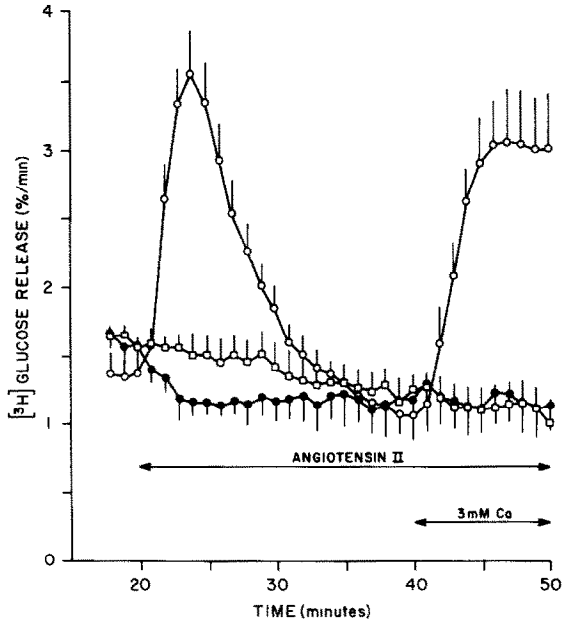

Fig.2. Effect of angiotensin II on [^{3}H]glucose release in the presence and absence of Ca. Cells perifused with zero-Ca medium 0–40 min and 3 mM Ca medium 40–50 min. 10^{-7} M angiotensin II present 20–50 min (○); 10^{-7} M sar^{1}ile^8-angiotensin II present 15–50 min and 10^{-7} M angiotensin II present 20–50 min (□); with no additions (●). Means and SEMs from 3–8 experiments.

presumably by a receptor-mediated mobilization of Ca. ATP (10^{-4} M) will also stimulate two phases of [^{3}H]glucose release; a transient phase in the absence of external Ca, and second phase when 3 mM Ca is present in the extracellular medium (fig.3, top). The Ca ionophore A23187 (10^{-6} M) gives similar results (fig.3, bottom). The transient increase in [^{3}H]glucose release in the absence of external Ca is probably due to the mobilization of intracellular Ca by A23187, which has been shown by several investigators in the liver [15–17]. The second phase of the response to ionophore, when Ca is returned to the perifusion medium, is most likely due to its generally accepted action of translocation of external Ca through the plasma membrane.

These results demonstrate that, as previously shown for the stimulation of ^{86}Rb efflux (a measure of K-release) [13], there are two phases in the stimulation of [^{3}H]glucose release (a measure of glycogenolysis) by Ca-mobilizing agonists. There is an initial transient phase of the response that is independent of extracellular Ca, which is probably a result of Ca released from an intracellular pool. The second phase of the response occurs only in the presence of extracellular Ca, which suggests that Ca influx is also involved in the mechanism of Ca-mobilization by these agents in the guinea pig hepatocyte. Authors in [18] found that rapid chelation of extracellular Ca with EGTA did not affect the initial rate and magnitude of the activation of phosphorylase in rat hepatocytes by α-agonists, vasopressin, and A23187. They concluded from this result that activation of phosphorylase by these agents does not involve in-

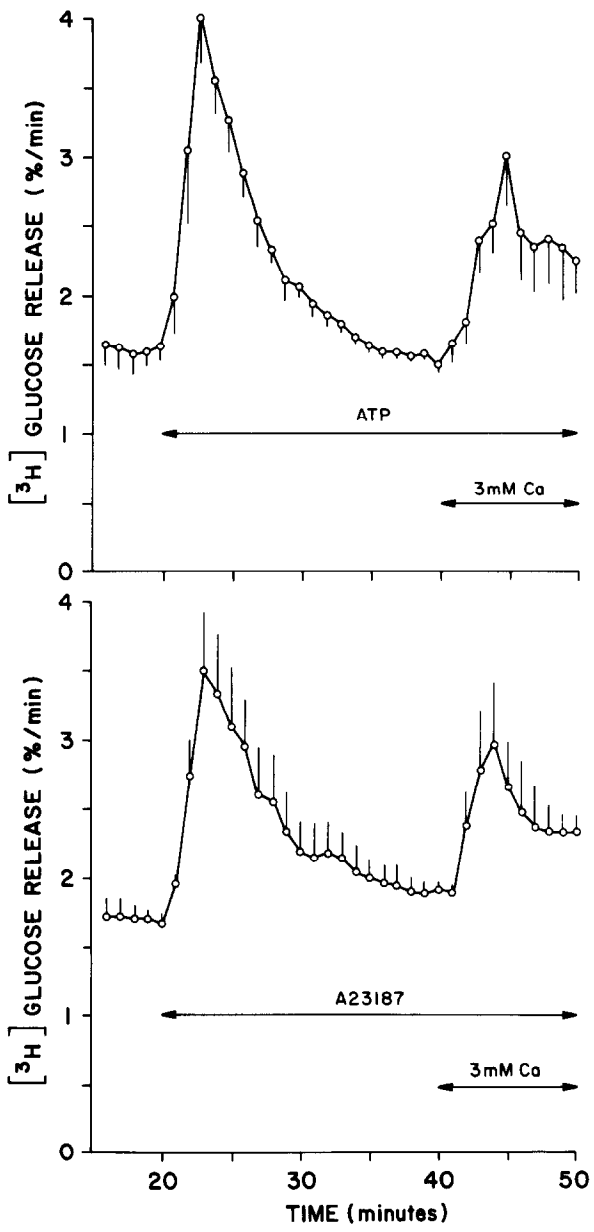

Fig.3. Effect of ATP and A23187 on [^{3}H]glucose release. Similar to protocol for fig.2. Upper panel, 10^{-4} M ATP present from 20–50 min; lower panel, 10^{-6} M A23187 (in 0.1% DMSO) present from 20–50 min. Means and SEMs from 3 experiments.

flux of external Ca, but that mobilization of intracellular Ca pools was sufficient to account for the response. It appears from our results that the initial rate and magnitude of the response are not particularly affected by the removal of external Ca, but that the duration of the response is dependent on the presence of extracellular Ca. In a medium that contains Ca, the relative importance of Ca-influx and Ca-release seems to depend on the length of time the agonist is present, with Ca influx playing more of a role as the duration of stimulation increases. The results presented here suggest that response of guinea pig hepatocytes to the cyclic AMP-independent agonists can be resolved into two phases. These phases presumably result from sequential or concomitant mechanisms involving Ca-release and Ca-influx.

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REFERENCES

[1] Keppens, S. and DeWulf, H. (1976) FEBS Lett. 68, 279–282.
[2] Keppens, S., Vandenheede, J.R. and DeWulf, H. (1977) Biochim. Biophys. Acta 496, 448–457.
[3] Hems, D.A., Davies, C.J. and Siddle, K. (1978) FEBS Lett. 87, 196–198.
[4] Garrison, J.C., Borland, M.K., Florio, V.A. and Twible, D.A. (1979) J. Biol. Chem. 254, 7147–7156.
[5] Saikai, K., Matsumura, S., Okimura, Y., Yamamura, H. and Nishizuka, Y. (1979) J. Biol. Chem. 254, 6631–6637.
[6] Cohen, P. (1980) in: Calcium and Cell Function (Cheung, W.Y. ed) vol.1, pp.183–199, Academic Press, New York.
[7] Whitton, P.D., Rodrigues, L.M. and Hems, D.A. (1977) Biochem. Soc. Trans. 5, 992–994.
[8] Hems, D.A., Rodrigues, L.M. and Whitton, P.D. (1978) Biochem. J. 172, 311–317.
[9] DeWulf, H. and Keppens, S. (1976) Arch. Int. Physiol. Biochim. 84, 159–160.
[10] Reinhart, P.H., Taylor, W.M. and Bygrave, F.L. (1982) Biochem. J. 208, 619–630.
[11] Williamson, J.R., Cooper, R.H. and Hoek, J.B. (1981) Biochim. Biophys. Acta 639, 243–295.
[12] Weiss, S.J. and Putney, J.W. jr (1978) J. Pharmacol. Exp. Ther. 207, 669–679.
[13] DeWitt, L.M. and Putney, J.W. jr (1983) J. Physiol., in press.

[14] Prpić, V., Blackmore, P.F. and Exton, J.H. (1982) J. Biol. Chem. 257, 11323–11331.
[15] Chen, J.L.J., Babcock, D.F. and Lardy, H.A. (1978) Proc. Natl. Acad. Sci. USA 75, 2234–2238.
[16] Blackmore, P.F., Brumley, F.T., Marks, J.L. and Exton, J.H. (1978) J. Biol. Chem. 253, 4851–4858.
[17] Blackmore, P.F. and Exton, J.H. (1981) Biochem. J. 198, 379–383.
[18] Blackmore, P.F., Hughes, B.P., Shuman, E.A. and Exton, J.H. (1982) J. Biol. Chem. 257, 190–197.