Rapid Stimulation by Vasopressin, Oxytocin and Angiotensin II of Glycogen Degradation in Hepatocyte Suspensions

By DOUGLAS A. HEMS, LORETA M. RODRIGUES and PATRICIA D. WHITTON
Department of Biochemistry, St. George's Hospital Medical School, Cranmer Terrace,
London SW17 0RE, U.K.

1. The hormonal control of glycogen breakdown was studied in hepatocytes isolated from livers of fed rats. 2. Glucose release was stimulated by [8-arginine]vasopressin (10 pM–10 nM), oxytocin (1 nM–1 μM), and angiotensin II (1 nM–0.1 μM). These responses are all at least as sensitive to hormone as is glucose output in the perfused rat liver. 3. The effect of these three hormones on glucose release was critically dependent on extracellular Ca^{2+}, unlike that of glucagon. Half-maximal restoration of the vasopressin response occurred if 0.3 mM-Ca^{2+} was added back to the incubation medium. 4. Glycogen breakdown was more than sufficient to account for the glucose released into the medium, in the absence or presence of hormones. Lactate release by hepatocytes was not affected by vasopressin, but was inhibited by glucagon. 5. If Ca^{2+} was omitted from the extracellular medium, vasopressin stimulated glycogenolysis, but not glucose release. 6. The phosphorylase *a* content of hepatocytes was increased by vasopressin, oxytocin and angiotensin II; minimum effective concentrations were 0.1 pM, 0.1 nM and 10 pM respectively. This response was also dependent on Ca^{2+}. 7. These results demonstrate that hepatocytes can respond to low concentrations of vasopressin and angiotensin II, i.e. these effects are likely to be relevant in the intact animal. The role of extracellular Ca^{2+} in the effects of these hormones on hepatic glycogenolysis and glucose release is discussed.

A range of hormones can promote glycogen breakdown in liver, the two best-established examples being glucagon and adrenaline. Other hormones that stimulate glycogenolysis, as inferred from stimulation of glucose release by perfused liver, include vasopressin (Hems & Whitton, 1973; Ma & Hems, 1975; Hems *et al.*, 1976) and angiotensin II (Hems *et al.*, 1976). Stimulation of glucose output by vasopressin has also been demonstrated in isolated hepatocyte suspensions (Stubbs *et al.*, 1976).

All hormones so far shown to stimulate hepatic glycogenolysis do so by increasing the amount of phosphorylase *a* in the tissue. This has been shown for vasopressin (Hems *et al.*, 1975, 1976; Keppens & De Wulf, 1975; Keppens *et al.*, 1977) and angiotensin II (Hems *et al.*, 1976; Keppens & De Wulf, 1976; Keppens *et al.*, 1977; Whitton *et al.*, 1977), as well as glucagon and adrenaline. Stimulation in liver cells of glucose output by vasopressin (Stubbs *et al.*, 1976) and of phosphorylase *a* content by vasopressin and angiotensin II (Keppens *et al.*, 1977; Whitton *et al.*, 1977) is critically dependent on the presence of extracellular Ca^{2+}, whereas cyclic AMP (Kirk & Hems, 1974; Keppens & De Wulf, 1975, 1976) or cyclic GMP (Hems *et al.*, 1978) do not appear to

be implicated in the hepatic effects of these two hormones.

Several questions raised by the above observations are taken further in the study described in the present paper, in which suspensions of hepatocytes have been used to clarify responses of carbohydrate metabolism to vasopressin, oxytocin, angiotensin II and, for comparison, glucagon. Glucose output in cell suspensions is shown to be stimulated by angiotensin II and oxytocin as well as by vasopressin, and the concentration-dependence of these hormone effects is documented. Also, stimulation of hepatocyte phosphorylase *a* activity in response to oxytocin is reported in the present study, and the concentration-dependence of this response in hepatocytes, so far described only for angiotensin II (Keppens & De Wulf, 1976), is presented for vasopressin and oxytocin. The data reveal a marked sensitivity of phosphorylase *a* in liver to these hormones. Quantitative relationships between glycogen breakdown and glucose release have been followed in response to vasopressin. Finally, the Ca^{2+}- and Mg^{2+}-dependence of hormone effects, and the role of extracellular Ca^{2+} in regulating the fate of glucose phosphates produced by glycogenolysis, have been investigated,

to shed light on those effects of hormones that involve extracellular Ca^{2+}.

Materials and Methods

Preparation of hepatocytes

Male Sprague–Dawley rats (200g) were fed on a Thompson's formula diet (Oxoid, London S.E.1, U.K.).

Isolated hepatocytes were prepared by perfusion of the liver with collagenase (Berry & Friend, 1969), as described by Krebs et al. (1974), with the following minor modifications: (i) hyaluronidase was omitted from the Ca^{2+}-free perfusion fluid, which contained 20mM-glucose to help maintain glycogen content, as well as collagenase [grade II; Boehringer Corp. (London), Lewes, Sussex, U.K.; 40mg in 80ml of perfusate]; (ii) after perfusion, the step of further shaking in a flask was omitted.

The yield of hepatocytes was 3–5g (from livers that normally weighed about 9g). Viability of hepatocyte preparations was assessed in several ways. The preparations of hepatocytes contained cells with macroscopically intact membranes, with almost no clustering. Trypan Blue was excluded by 90–95% of cells. In preparations made by the standard procedure from livers of starved rats, rates of gluconeogenesis from lactate were similar to those reported by other laboratories (e.g. Krebs et al., 1974). The glycogen content of cells was equivalent to that in vivo (200–300μmol of glucose/g wet wt. of liver). Finally, sensitivity of glycogen breakdown to hormones at lower concentrations than in the perfused liver (Hems et al., 1976), and to glucagon over the range 10pM–1nM (results not shown), was taken as evidence of the satisfactory viability of preparations of cells.

Cells were incubated for 40min at 37°C in a shaking bath in plastic vials, in 2ml of bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 2% (w/v) bovine serum albumin (fraction V; Miles Laboratories, Stoke Poges, Bucks., U.K.). All measurements were made in duplicate vials. Incubations, containing about 12mg (dry wt.) of cells, were terminated with 0.2ml of 20% (w/v) $HClO_4$.

The time course of glucose release by cells was approximately linear. During 40min a gradual and slight decrease in the rate of release was observed; hormones did not affect this characteristic, although they stimulated release. In view of this fact, data are presented as changes during 40min, rather than as rates.

To measure glucose output or O_2 uptake in the perfused liver, the perfusion technique described by Hems & Whitton (1973) was used.

Analytical methods

Methods for measuring glycogen, glucose and lactate have been described (Hems & Whitton, 1973). Glycogen in hepatocytes was isolated after boiling in 15% (w/v) KOH, and precipitation with saturated Na_2SO_4 and 3vol. of ethanol.

In one group of experiments, glycogen phosphorylase a activity was assayed in cells. For these experiments, cells were prepared as usual, but without glucose in the perfusate, and were incubated in the presence of 20mM-glucose. The initial phosphorylase a activity was 57.6 ± 6.0 (5) μmol/min per g dry wt. of cells (mean$\pm$S.E.M.), which fell during 30min incubation at 37°C to 18.4 ± 2.9. Hormones were then added to cell suspensions, and the gas phase was quickly flushed with O_2/CO_2 (19:1), before reclosing the vial. The incubations were stopped 2min after addition of the hormone by rapidly freezing 0.5ml of incubation medium in plastic centrifuge tubes in liquid N_2. Hepatocytes were homogenized after addition of 2vol. of an ice-cold medium containing 75mM-glycylglycine and 150mM-NaF, pH7.0. Glycogen phosphorylase a activity was assayed by following incorporation of [^{14}C]glucose 1-phosphate into glycogen at 30°C and pH6.5 as described by Hems et al. (1976).

Chemicals

Chemicals were of the highest grade commercially available. [8-Arginine]vasopressin and oxytocin were the highest grade made by Sigma (London) Chemical Co. (Kingston upon Thames, Surrey, U.K.), and synthetic [5-valine]angiotensin II was kindly provided by the Medical Research Council National Institute of Biological Standards (Hampstead, London N.W.3, U.K.). Glucagon (insulin-free) was kindly given by Eli Lilly, Indianapolis, IN, U.S.A.

Results

Concentration-dependence of hormonal stimulation of glucose output

The sensitivity of hepatocytes to stimulation of glucose output by vasopressin, angiotensin II (Fig. 1) and oxytocin (Fig. 2) was measured. The concentration–response curves may be compared with the sensitivity to hormones of the perfused liver of rats of the same age, strain and sex (Hems et al., 1976): glucose output in hepatocytes (Fig. 1) exhibited about a 5-fold greater sensitivity than the perfused liver to vasopressin, but no greater sensitivity to angiotensin II. To permit comparison of the sensitivity of oxytocin of the perfused liver with that of hepatocytes, the potency of the effect of oxytocin on the perfused liver (not previously reported) was established (Fig. 2); hepatocytes showed sensitivity to oxytocin of the same order as that of the perfused liver.
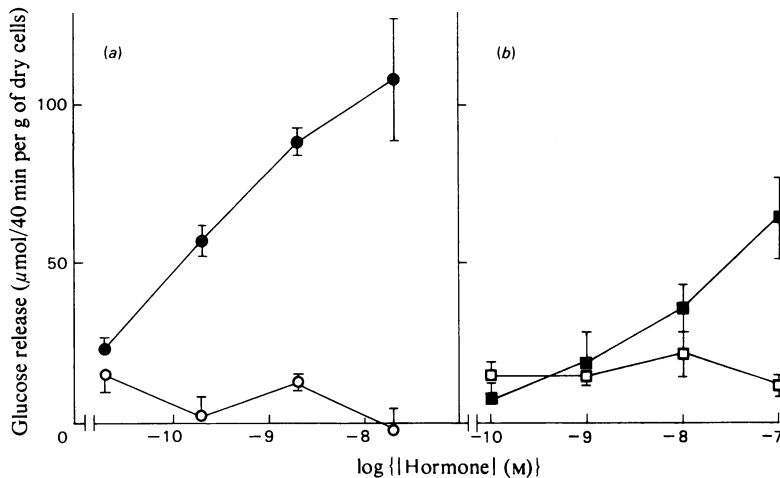
Fig. 1. *Concentration-dependence and role of Ca^{2+} in hormonal stimulation of glucose release by hepatocytes*
Hepatocytes were prepared in the usual way, except that in some preparations cells were washed and incubated in Ca^{2+}-free medium ($\bigcirc$, $\square$). Cells were incubated for 40 min in standard ($\bullet$, $\blacksquare$) or Ca^{2+}-free ($\bigcirc$, $\square$) medium, with either (a) vasopressin or (b) angiotensin II. Glucose release (μmol/40 min per g of dry cells) is expressed as the increase over the control value for each preparation (130–150 μmol). The initial glycogen content was about 800 μmol of glucose/g of dry cells. Other details are in the text. Results are means from three preparations, with bars indicating the s.e.m.
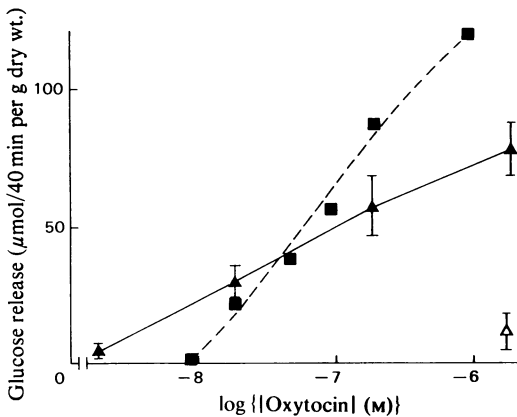

Fig. 2. *Concentration-dependence of stimulation of glucose release by oxytocin*
Glucose release was measured over 40 min in response to oxytocin, either in the perfused rat liver ($\blacksquare$) as described by Hems & Whitton (1973), or in hepatocytes incubated in standard ($\blacktriangle$) or Ca^{2+}-free ($\triangle$) medium as described in Fig. 1. Results are expressed per g of dry whole perfused liver, or per g of dry cells (over control values). Values are either from individual perfusions ($\blacksquare$) or are means±s.e.m. (bars) from three cell preparations.

Ca^{2+}-dependence of hormonal stimulation of glucose output

Previous experiments have established that the effect of vasopressin on hepatic glucose output is critically dependent on the presence of extracellular

Ca^{2+} (Stubbs *et al.*, 1976; Whitton *et al.*, 1977). This observation was expanded in the present investigation. Throughout the range of effective vasopressin and angiotensin II concentrations omission of Ca^{2+} from the incubation medium prevented the hormone effects on glucose output almost completely (Fig. 1). In experiments in which $CaCl_2$ was added back to hepatocytes during incubation, the concentration-dependence of the cofactor role of extracellular Ca^{2+} in vasopressin action was established. Cells were quite sensitive to Ca^{2+}, a half-maximal vasopressin effect on glucose output being restored at about 0.3 mM-Ca^{2+}. The Ca^{2+}-dependence of the action of oxytocin on glucose output was as marked as that of vasopressin (Fig. 2).

In view of the crucial role of extracellular Ca^{2+} in the effects of vasopressin and angiotensin II on hepatic glucose output, the Mg^{2+}-dependence of this effect was investigated in experiments where Mg^{2+} was omitted from the medium in which hepatocytes were washed, and then either omitted also from the incubation medium or added back during incubation. No effect of Mg^{2+} omission was observed on glucose output with either hormone (results not shown).

Effects of hormones on metabolism of glycogen and lactate

Changes in hepatocyte glycogen content and in lactate and glucose concentrations in the incubation fluid were followed. Data are presented as net metabolic changes during a 40 min incubation (Table

Table 1. *Effects of hormones and EGTA on carbohydrate metabolism in hepatocytes, calculated as changes during incubation*

Hepatocytes were prepared and incubated in the standard manner; in some preparations, cells were washed and incubated in Ca^{2+}-free medium. Cells were incubated for 40min, and metabolic responses are calculated as changes over initial values: glucose and lactate concentrations respectively were about 1.5 and 0.5mM initially, and initial glycogen content of cells was 800–1100μmol of glycogen glucose/g of dry cells. Additions to the medium were: vasopressin (20nM); glucagon (1 μM); EGTA (2.5mM). Other details are in the text. Results are means±s.e.m. of five measurements (and five cell preparations), except that there were four in the experiments with glucagon. *$P<0.02$, cf. appropriate hormone-free control (two-tailed t test); †$P<0.05$, cf. Ca^{2+}-containing control.

	Metabolic changes (μmol/40min per g of dry cells)			
	Release			Breakdown of glycogen
Additions	Glucose	Lactate	Glucose plus (lactate)/2	
Incubations with Ca^{2+}				
Control	193±17	153±17	270±15	−304±36
Vasopressin	279±22*	167±24	363±18	−470±16*
Glucagon	489±21*	−31±20*	475±29	−558±44*
EGTA	182±13	67±25†	216±16	−374±20
Vasopressin plus EGTA	188±10	85±23	231±13	−387±20
Incubations Ca^{2+}-free				
Control	148±16	105±16	200±21†	−342±38
Vasopressin	137±23	111±20	192±24	−413±45
Glucagon	375±9*	8±15*	379±15	−617±48*

1). Both glucose and lactate were released throughout all incubations, except in the presence of glucagon, where net lactate uptake was observed. In the absence of hormones, and in standard Krebs–Ringer bicarbonate-buffered saline, glycogen breakdown accounted for all of the lactate and glucose produced by the hepatocytes (Table 1).

The effect of depletion of Ca^{2+} in the extracellular fluid was tested on these processes, by either omitting Ca^{2+} from, or adding EGTA to, the incubation fluid. Both manipulations decreased the release of lactate, and omission of Ca^{2+} also decreased the glucose release (Table 1). Neither manipulation diminished the amount of glycogen degraded during incubation (Table 1).

Effects of hormones on metabolism of glycogen and lactate were also tested. Vasopressin was selected as representative of the group of hormones that do not act via cyclic monophosphates of purine nucleosides, and its effects were compared with those of glucagon (Table 1). In normal conditions (i.e. including extracellular Ca^{2+}) vasopressin stimulated glucose output, but not lactate release. Glucagon, in contrast, markedly inhibited lactate release; rather, lactate uptake was observed (Table 1) despite the low initial lactate concentration (about 1.0mM). In the absence of Ca^{2+}, glucagon caused glycogenolysis, and release of glucose, to virtually the same extent as in the presence of Ca^{2+} (Table 1). In the absence of extracellular Ca^{2+}, no extra release of glucose or lactate occurred in response to vasopressin (over the relevant control value; Table 1). In the presence or absence of Ca^{2+} or hormones,

degradation of glycogen was sufficient to account for glucose or lactate released.

For statistical assessment of effects of hormones in hepatocytes, it is advantageous to compare changes during incubation with control values for the same cell preparation. Further insight into the data of Table 1 may be gained in this way. The extra glycogen breakdown caused by vasopressin was 166±29 (5) μmol of glycogen glucose/g of dry cells (mean±s.e.m.) in 40min, in the presence of Ca^{2+} (over the paired control values: $P<0.02$), and 71±16 (5) in the absence of Ca^{2+}. Thus, in the absence of added extracellular Ca^{2+}, vasopressin caused less extra glycogenolysis (over control values, compared with values in the presence of Ca^{2+}), although some stimulation (about 40%) still occurred.

Effect of vasopressin on O_2 uptake

In the experiments described above, vasopressin (and glucagon) consistently stimulated glycogen breakdown by an amount that was more than sufficient to account for glucose or lactate released. One possible explanation for this observation is that oxidation of glycogen-derived carbon to CO_2 was stimulated by vasopressin. Therefore O_2 uptake was measured in perfused livers. O_2 concentration in input and effluent perfusate was measured with an oxygen electrode (Radiometer, Copenhagen, Denmark) and the total O_2 content was calculated from the solubility coefficient and the oxygen-dissociation curve for rat erythrocytes (Gray & Steadman, 1964). The rate, 3.75±0.14 (3) μmol/min per g (mean± s.e.m.) in control perfusions, was increased immedi-

ately on addition of 20nM-vasopressin to the per-fusate, by 0.89 ± 0.05 (3). This increase occurred within 2min of hormone addition, and then O_2 uptake slowly decreased to the previous rate, during the subsequent 10–30min (results not shown).

Effect of hormones on phosphorylase a activity

Vasopressin or angiotensin II increased the amount of phosphorylase a in hepatocytes (measured 2min after hormone addition; Fig. 3); this response was more sensitive to hormones than that of glucose output (measured over 40min; Fig. 1). The minimum effective concentrations of hormones that stimulated phosphorylase a activity in cells were about 0.1pM-vasopressin, 10pM-angiotensin II and 0.1nM-oxy-tocin (Fig. 3).

The role of extracellular Ca^{2+} in hormonal stimulation of phosphorylase a activity in hepatocyte suspensions was tested previously (Whitton et al., 1977), when it was observed that omission of extracellular Ca^{2+} decreased this response, but did not abolish it. A more severe Ca^{2+} depletion was achieved in the present experiments by incubating cells in the absence of Ca^{2+} and plus EGTA. The

increases in phosphorylase a caused by hormones were abolished by EGTA (Fig. 3). This in general confirms the findings of Whitton et al. (1977) and Keppens et al. (1977) for the effect of angiotensin II and vasopressin on phosphorylase, and is the first documentation of the Ca^{2+}-dependence of the stimulation of phosphorylase a activity by oxytocin.

Discussion

Stimulation of glycogen degradation and glucose output by hormones

The present experiments demonstrate that vaso-pressin, oxytocin and angiotensin II can stimulate glucose output, and increase the amount of phos-phorylase a, in rat hepatocyte suspensions. This confirms and extends previous reports of the effect (in hepatocytes) of vasopressin on glucose output (Stubbs et al., 1976) and of vasopressin and angioten-sin II on phosphorylase a activity (Keppens & De Wulf, 1975, 1976; Whitton et al., 1977; Keppens et al., 1977).

The most potent effect of hormones on hepato-cytes was to increase the amount of phosphorylase a. This response was measured in cells only 2min after addition of hormone, so the observed concentration-dependence of this effect cannot have been affected by hormone destruction during this period. With vasopressin and angiotensin II, this response (minimum effective concentrations being about 0.1 and 10pM respectively) was more sensitive than in the perfused liver (minimum effective concen-trations about 50pM; Hems et al., 1976). The sensitivity of hepatocyte phosphorylase to acti-vation by vasopressin in particular is exquisite. The minimum effective concentration (0.1pM, i.e. about 100fg/ml) is lower than the concentration in plasma (in any condition). In our experiments the concen-tration of vasopressin molecules was of the same general order as the concentration of hepatocytes, implying that only a few molecules of vasopressin may suffice to activate glycogenolysis in a single liver cell.

The concentration-dependence of the stimulatory effect of vasopressin, oxytocin and angiotensin II on hepatic glucose output, established in suspensions of hepatocytes, is also likely to reflect the true sensitivity of parenchymal cells to the hormones. Cells were incubated in dilute suspensions (about 30mg wet wt./ml), so that hormone destruction is unlikely to have been significant. Also, hepatocyte suspensions are at least as sensitive to the hormonal stimulation of glucose output as is the perfused liver; this emerges if the responses to angiotensin II and vasopressin by perfused liver, followed over 5min by Hems et al. (1976), are compared with the response of
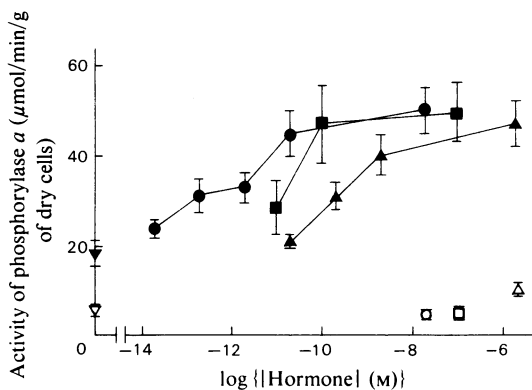

Fig. 3. *Concentration-dependence and role of Ca^{2+} in hormonal stimulation of the content of phosphorylase a in hepatocytes*
Hepatocytes were prepared in the usual way, except that in some preparations cells were washed in Ca^{2+}-free medium (open symbols). Cells were incu-bated in the standard medium or Ca^{2+}-free medium containing 2mM-EGTA; both media contained 20mM-glucose. After 30min hormones at various concentrations were added. Phosphorylase a (μmol of glucose transferred/min per g of dry cells) was assayed in normal and Ca^{2+}-free medium (closed and open symbols respectively) 2min after addition of either vasopressin (●, ○), angiotensin II (■, □) or oxytocin (▲, △), and in control incubations (▼, ▽). Results are means±S.E.M. of three to five cell preparations.

cells. Hence it is unlikely that significant hormone destruction has decreased the apparent sensitivity to hormones in hepatocyte suspensions. Inasmuch as any such destruction occurred, the present response curves would represent an underestimate of potency of hormones.

The effect of oxytocin to stimulate hepatic glucose release (or to inhibit glycogen synthesis; Whitton & Hems, 1976) is about 1000-fold less potent than that of [8-arginine]vasopressin, showing that in vasopressin the 3-phenylalanine and 8-arginine residues are crucial to the effect on hepatic glycogenolysis.

Free glucose constitutes only one fate of glycogen-derived carbon in liver, so it is relevant to evaluate the quantitative relationships between the amount of glycogen degraded, and its products, in glycogenolytic states. The data suggest that vasopressin may stimulate degradation to produce glucose phosphates for assimilation within the liver, rather than for release as free glucose. Thus stimulation of glycogen degradation by vasopressin was more than sufficient to account for the extra glucose released, confirming previous experiments with vasopressin in the perfused liver (Hems & Whitton, 1973). Clearly, lactate is not the extra product derived from hormone-induced glycogen breakdown, as its release was not affected by vasopressin. However, pyruvate can meet other fates; it is possible that stimulation of glycogen-supported glycolysis can occur in response to vasopressin and hormones of this group (i.e. those not acting via cyclic purine nucleotide monophosphates), at least in some conditions in rat liver.

The functional significance of these hormone effects may be evaluated by comparing hormone concentrations in blood in the intact animal with those that can affect the liver. The potency of the effects of both vasopressin and angiotensin II on hepatocytes strongly suggests that these two hormones can act on liver glycogen in the intact animal, e.g. in various stress or adaptive states (see discussion by Hems et al., 1976). The minimum effective concentration of oxytocin (about 0.1 nM for the stimulation of phosphorylase a) is of the same order as the highest concentration of oxytocin so far reported in blood of adult rats (up to about 0.1 nM, during parturition; M. Forsling, unpublished work). Thus oxytocin action on the liver may sometimes be operative *in vivo*.

Role of Ca^{2+} in hormonal control of hepatic glycogenolysis

The experiments described here confirm that there is a critical requirement for extracellular Ca^{2+} in the stimulation in hepatocytes of glucose output by vasopressin (Stubbs et al., 1976) and of phosphorylase a activity by vasopressin or angiotensin II (Whitton et al., 1977; Keppens et al., 1977). The present experiments extend these observations in

demonstrating that the same is true for the stimulation of glucose output by angiotensin II and oxytocin. Also the present results show that high concentrations of hormones do not overcome the impairment of effects that occurs in Ca^{2+}-deficient media.

Stimulation of glucose output in hepatocytes by vasopressin, oxytocin and angiotensin II is associated with an increase in the amount of available phosphorylase a in hepatocytes. Thus their action in cells must involve either activation of phosphorylase b kinase or inhibition of phosphorylase a phosphatase. With glucagon, for example, the major mechanism is that phosphorylase b kinase is activated by phosphorylation (Van de Werve et al., 1977; Keppens et al., 1977). However, vasopressin and angiotensin II do not increase the content of cyclic AMP or cyclic GMP in liver (Kirk & Hems, 1974; Hems et al., 1978 or bring about activation of protein kinase (Keppens et al., 1977). Since Ca^{2+} is critically implicated in their glycogenolytic effect, and since phosphorylase b kinase is activated by Ca^{2+} (Khoo & Steinberg, 1975; Van de Werve et al., 1977), one possibility is that phosphorylase b kinase is activated by Ca^{2+} in response to the hormones, as has been suggested for α-adrenergic stimulation of hepatic glycogenolysis (Keppens et al., 1977; Van de Werve et al., 1977; Assimacopoulos-Jeannet et al., 1977).

The Ca^{2+} status of the hepatocyte can influence the fate of glycogen-derived carbon. In the experiments where Ca^{2+} was omitted, or EGTA added (in the absence of hormones), lactate release was significantly decreased, although glycogen breakdown was not diminished. Glucose release was also less in the absence of extracellular Ca^{2+}. Thus extracellular Ca^{2+} can influence the fate of glycogen-derived carbon within the liver (perhaps via parallel changes in an intracellular Ca^{2+} pool) by promoting glucose release, or lactate release in the presence of low extracellular lactate concentrations. In the presence of added lactate, extracellular Ca^{2+} promotes gluconeogenesis in hepatocytes (Elliott, 1976). These observations imply that, when lactate and Ca^{2+} are both present in the extracellular phase (as in most situations in the animal), carbon of the glycogen-derived hexose and glycolytic-intermediate pools is directed towards release as glucose.

Glycogen breakdown was stimulated by EGTA, implying that the availability of Ca^{2+} to phosphorylase b kinase is not critical during basal glycogenolysis. In the presence of EGTA, no further glycogenolysis or stimulation of phosphorylase a was produced by vasopressin. This impairment of the vasopressin effect, by EGTA, was more severe than that produced by merely omitting Ca^{2+} from the incubation medium, which decreased, but did not abolish, either the phosphorylase a activation

(Whitton *et al.*, 1977) or the glycogenolysis observed with vasopressin (the present work). Thus the stimulation of glycogen breakdown and phosphorylase activity by vasopressin is controlled by Ca^{2+} availability within the cell, unlike hormone-stimulated glucose release, which is critically dependent on the presence merely of extracellular Ca^{2+}. This difference in Ca^{2+}-dependence could imply the existence of a specific role of Ca^{2+} at the outer cell membrane in the stimulation of hepatic glucose release by vasopressin and angiotensin II, so that, in the absence of extracellular Ca^{2+}, glycogenolysis induced by these hormones supplies carbon solely for intracellular fates.

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