Glycogen Phosphorylase, Glucose Output and Vasoconstriction in the Perfused Rat Liver

CONCENTRATION-DEPENDENCE OF ACTIONS OF ADRENALINE, VASOPRESSIN AND ANGIOTENSIN II

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1. Glycogen phosphorylase (α form, in rapidly freeze-clamped samples) and glucose release were measured in the perfused liver, in response to a range of concentrations of adrenaline, [8-arginine]vasopressin (anti-diuretic hormone) and angiotensin II. 2. All three hormones increased phosphorylase α activity by about $10\mu\text{mol/min per g}$ of fresh liver, which was more than sufficient to explain concomitant glucose release ($1\text{--}2\mu\text{mol/min per g}$). 3. Minimally effective concentrations which activated phosphorylase were: adrenaline, 10 nM (2 ng/ml); vasopressin, 40 pM (40 pg/ml , $15\mu\text{units/ml}$); angiotensin II, 60 pM (60 pg/ml). 4. Glycogen synthase activity was inhibited by adrenaline and vasopressin but not significantly by angiotensin II. 5. Vasoconstriction observed with adrenaline and angiotensin II (but not vasopressin) might explain part of the activation of phosphorylase, since equivalent vasoconstriction (in separate perfusions) activated phosphorylase, but did not stimulate glucose output or inhibit synthase. 6. The potency of these effects suggests that all three hormones can stimulate hepatic glycogen degradation *in vivo* (by direct hepatic action). It is proposed that hormones, and ischaemia, stimulate glycogen degradation to provide glucose phosphates for disposal within the liver cell, as well as for release as free glucose.

Short-term stimulation of hepatic glycogen degradation can be caused by a number of hormones, as shown most clearly in experiments with the perfused liver. The effect of glucagon, mediated via cyclic AMP, is well established (Glinsmann & Mortimore, 1968; Mackrell & Sokal, 1969; Exton *et al.*, 1971). Similarly, adrenaline stimulates glycogenolysis, as measured by glucose output or activation of glycogen phosphorylase (Exton *et al.*, 1971; Saitoh & Ui, 1975, 1976); contrary to earlier views, at least one major route of adrenaline action does not involve cyclic AMP (Tolbert *et al.*, 1973; Tolbert & Fain, 1974; Saitoh & Ui, 1976). Finally, vasopressin can stimulate glycogenolysis in the perfused liver of the rat (Hems & Whitton, 1973) and mouse (Ma & Hems, 1975). This effect, in rats at least, is not mediated by cyclic AMP (Kirk & Hems, 1974; Keppens & de Wulf, 1975).

Several problems remain unsettled in regard to these effects. In response to vasopressin, an increase in the amount of hepatic glycogen phosphorylase α has been reported only in the intact rat (Keppens & de Wulf, 1975; Hems *et al.*, 1975). This does not constitute definitive evidence for a direct hormone action on the liver, and could, for example, be a consequence of vasoconstriction (perhaps in the gut).

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Adrenaline, like vasopressin, is a powerful pressor agent (in the intact animal), so vasoconstriction could be implicated in adrenaline-dependent hepatic glycogenolysis. Such metabolic effects, observed *in vivo*, provide no information about the sensitivity of the liver to a hormone, even if direct hepatic action is implicated. A further area of uncertainty arises because glucose output need not closely parallel the activation of glycogen phosphorylase, in view of the existence of alternative fates for glycogen-derived glucose.

Another vasoactive agent which has been reported to cause hyperglycaemia, in rabbits (Akinkugbe *et al.*, 1967), dogs (Heidenreich & Kook, 1963) and man (Ajzen *et al.*, 1968) is angiotensin II, the peptide produced as a result of renin action (for reviews, see Laragh & Sealey, 1973; Regoli *et al.*, 1974). The biochemical effects of angiotensin II have not been well documented (Allman, 1974). Angiotensin II is a potent pressor agent, so its effect on blood glucose concentration is subject to the same doubts as those described above.

As an approach to these problems, the concentration-dependence of the actions of vasopressin, adrenaline and angiotensin II on glycogen phosphorylase α activity has been investigated in the perfused rat liver. Glucose output and blood-flow rates have also been measured to assess their relationships to phos-

phorylase activity. The present results provide further evidence that hepatic glycogen breakdown is activated by a variety of hormones.

Materials and Methods

Livers of fed male Sprague-Dawley rats (180 g) were perfused with 50 ml of bicarbonate-buffered saline containing albumin and washed rat erythrocytes, gassed with $O_2 + CO_2$ (95:5) (Hems & Whitton, 1973). The glucose concentration, initially 5 mM, stabilized at 8–10 mM. After 40 min, hormones were added (as a single addition in all experiments). After a further 4 min, simultaneous samples of input and output blood were taken for glucose determination by a glucose oxidase method (Trinder, 1969), in triplicate. Liver samples were removed and immediately freeze-clamped, at the temperature of liquid N_2 ; a control sample was removed after 38 min (half of median lobe) and the second sample at 45 min, i.e. 5 min after hormone (left lateral lobe). General aspects of this serial-sampling procedure have been described (Hems *et al.*, 1972; Whitton & Hems, 1975, 1976).

In control perfusions, samples were taken as described above, but no hormone was added at 40 min. In one group of perfusions, the vasoconstriction produced by hormones was simulated (with regard to time-course and extent) by lowering the pressure of the input perfusate; samples were removed as usual. Perfusate flow rate was measured with a small calibrated (5 ml) glass siphon device, inserted in the apparatus to receive dripping effluent medium.

Glycogen phosphorylase (α form) and synthase (α and total activities) were assayed in non-centrifuged homogenates, by using ^{14}C -labelled substrates, essentially as described by Das & Hems (1974) with minor modifications (Whitton & Hems, 1976; Stalmans & Hers, 1975). The mixture used in the phosphorylase α assay contained glycogen (1%, w/v), [^{14}C]glucose 1-phosphate (50 mM), EDTA (10 mM), NaF (150 mM) and caffeine (0.5 mM), but not AMP (Stalmans & Hers, 1975); the final pH was 6.5. The synthase assay mixture (Whitton & Hems, 1976) for the α form included Na_2SO_4 (10 mM), and that for the total activity included glucose 6-phosphate (7 mM). There are no differences in phosphorylase or synthase activity between lobes sampled simultaneously (Whitton & Hems, 1975); this validates the use of sequentially removed liver samples.

Sources of hormones were as follows: Adrenaline bitartrate and [8-arginine]vasopressin (synthetic, grade VI) were from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.). Angiotensin II (synthetic [5-valine] analogue; batch 64/15; 950 units/mg) was kindly provided by the National Institute for Biological Standards (Medical Research Council), Hampstead, London N.W.3, U.K. Vasopressin and angiotensin II were used fresh or stored frozen in 50 mM-sodium acetate (pH 4.8) and

used after one thaw. Adrenaline was dissolved in water and used fresh; a 100 μ l portion or less was added to perfusate. Other chemicals were of the highest grade available.

Results

The effects of single additions of hormones (at 40 min) on glycogen phosphorylase α activity (after 5 min), net glucose output (after 4 min) and perfusate flow rate (2–4 min after hormone) are presented in Figs. 1–3. In livers perfused in the present conditions, the glycogen content after 40 min is 300–400 μ mol of glucose/g of fresh liver (Hems & Whitton, 1973).

Action of vasopressin on the liver

In response to vasopressin, glycogen phosphorylase was activated by concentrations in the range 40 pM–1 nM, i.e. about 15–400 μ units/ml, or about 40–1000 pg/ml. Glucose output did not occur at the lowest of these concentrations, but only in response to concentrations greater than 0.1 nM-vasopressin (Fig. 1). Throughout this dose range, no vasoconstriction occurred; only at concentrations greater than 10 nM did moderate decreases in blood flow occur (Fig. 1).

Action of adrenaline on the liver

Glucose output was stimulated by adrenaline, over the concentration range 10–200 nM (Fig. 2), in approximate agreement with previous results (Exton *et al.*, 1971; Saitoh & Ui, 1976). Glycogen phosphorylase was activated by similar concentrations of adrenaline (Fig. 2). Vasoconstriction occurred over the range 30–300 nM, i.e. the minimally effective concentration was rather greater than that which activated phosphorylase.

Action of angiotensin II on the liver

The effects of angiotensin II on the liver (Fig. 3) resembled those of adrenaline, particularly in that significant vasoconstriction was observed. Activation of glycogen phosphorylase and vasoconstriction occurred over approximately the same dose range (0.1–10 nM). Angiotensin II also produced glucose output (minimally effective concentration about 0.5 nM), and the maximal output resembled that produced by adrenaline or vasopressin (about 2 μ mol/min per g).

Actions of hormones on glycogen synthase

There are complex inter-relations between the activities of the glycogen phosphorylase and synthase enzymes in liver (Stalmans *et al.*, 1974; Hers *et al.*, 1974). Therefore the synthase response was also assessed in response to concentrations of hormones which brought about near-maximal effects on phosphorylase (Figs. 1, 2 and 3). Adrenaline caused inhibition of synthase activity (i.e. a diminished percentage of form α ; Table 1), as has been reported previously (Saitoh & Ui, 1975). Vasopressin brought

Fig. 1. Action of [8-arginine]vasopressin on the liver

Livers of fed rats were perfused with bicarbonate-buffered saline. A single addition of hormone was made after 40 min. Measurements were made (a) of the increase in phosphorylase *a* activity after a further 5 min (■) and of blood-flow rate (3 min after hormone, ●), and (b) of glucose output after 4 min (▲). The initial (control) phosphorylase activity was about 7 μ mol of glucose/min per g of fresh liver (see Table 2). Hormone concentrations were corrected for the variation in perfusate volume, and groups of results with similar concentrations have been combined: this accounts for the variation (indicated by bars) in the horizontal plane. For other details, see the text. Results are means of three or four values; vertical bars indicate the S.E.M.

about a less marked diminution in the activity of synthase *a*, in keeping with its lack of effect on synthase *in vivo* (Whitton, 1975).

Effects of partial ischaemia in the perfused liver

In perfusions with either adrenaline or angiotensin II, vasoconstriction occurred over about the same range of concentrations of hormone as that which stimulated glucose output and phosphorylase activity; the latter effects could therefore have been due to hypoxia in the liver, produced by diminution in blood flow. This was tested in experiments where the input pressure was decreased, to diminish the blood flow through the perfused liver by the same extent as that brought about by the saturating concentrations of hormones (i.e. by about 50% between 2 and 4 min after the start of lower pressure). In these conditions, there was significant activation of glycogen phosphorylase, but no stimulation of glucose output (Table 2). Also, glycogen synthase activity was not altered; thus changes caused by hormones (and by adrenaline in particular; Table 1) were not due to attendant ischaemia.

Discussion

Activation of glycogen phosphorylase by hormones

The present studies involved measurements of phosphorylase activity in liver samples which were rapidly frozen within 1–2 s. Such speed of fixation is crucial in studies of this enzyme (Stalmans *et al.*, 1974). The assay conditions were specific for the *a* form (see Stalmans & Hers, 1975), partly because the *b* form exhibits a low affinity for glucose 1-phosphate (Tan & Nuttall, 1975). Thus the observed changes in activity indicate the conversion of the *b* into the *a* form.

Activation of hepatic glycogen phosphorylase by vasopressin can occur in intact rats (Hems *et al.*, 1975; Keppens & de Wulf, 1975). The response of phosphorylase in the perfused liver confirms that this enzyme responds to direct hepatic action of vasopressin, and is very sensitive to the hormone (minimally effective concentration 40 pM, i.e. about 40 pg/ml or 15 μ units/ml).

Direct action of adrenaline on liver glycogen metabolism has been thought to be unimportant *in vivo*, as a result of studies in the perfused liver of the rela-

Fig. 2. Action of adrenaline on the liver

Livers were perfused for 40 min, and then responses to various concentrations of adrenaline were measured. Details are as described in Fig. 1.

Fig. 3. Action of angiotensin II on the liver

Livers were perfused for 40 min, and then responses to various concentrations of angiotensin II were measured. Details are as described in Fig. 1.

Table 1. *Influence of hormones on glycogen synthase activity*

Livers were perfused as described in Figs. 1, 2 and 3 and in the text. Hormones were added after 40 min. Glycogen synthase was assayed in one sample after 38 min (control value before hormones) and in a second sample 5 min after hormones. The initial concentrations of hormone were: adrenaline, 1.1 μ M; angiotensin II, 3.5 nM; vasopressin, 0.18 nM. Results are means $\pm$ S.E.M. of three measurements. The significance of hormone effects is shown by the *P* values, obtained by the *t* test for significance of differences between related mean values (of % *a*).

Hormone	Glycogen synthase (μmol of glucose/min per g of fresh liver)						<i>P</i> value
	Control			After hormone			
	Form <i>a</i>	Total	<i>a</i> (%)	Form <i>a</i>	Total	<i>a</i> (%)	
Adrenaline	0.24 $\pm$ 0.01	0.70 $\pm$ 0.04	35 $\pm$ 3	0.12 $\pm$ 0.02	0.64 $\pm$ 0.05	19 $\pm$ 2	<0.01
Angiotensin II	0.27 $\pm$ 0.02	0.82 $\pm$ 0.01	33 $\pm$ 3	0.20 $\pm$ 0.02	0.70 $\pm$ 0.06	29 $\pm$ 3	>0.20
Vasopressin	0.34 $\pm$ 0.04	0.81 $\pm$ 0.07	42 $\pm$ 2	0.25 $\pm$ 0.03	0.80 $\pm$ 0.04	31 $\pm$ 3	<0.05

Table 2. *Effects of ischaemia on enzymes and glucose output*

Livers were perfused for 40 min and then ischaemia was produced by decreasing the pressure of input medium. The time-course of the change in flow rate was designed to simulate the vasoconstriction produced by saturating concentrations of adrenaline or angiotensin II (see the text). The flow rate was decreased by about 50% during 2 min, and then kept steady for 3 min; samples of medium were taken after 4 min of ischaemia. Enzymes in liver samples were assayed after 38 min (control value) and 45 min. Results ($\pm$ S.E.M.) are means of the number of measurements indicated in parentheses. The response of phosphorylase to ischaemia is significant (*P* < 0.05); the other differences are not significant.

	Control	Ischaemia
Phosphorylase (μ mol of glucose/min per g)	6.9 $\pm$ 0.8 (5)	11.6 $\pm$ 1.4 (5)
Glycogen synthase (μ mol of glucose/min per g):		
Form <i>a</i>	0.35 $\pm$ 0.02 (4)	0.36 $\pm$ 0.03 (4)
Total	0.87 $\pm$ 0.09 (4)	0.95 $\pm$ 0.09 (4)
Form <i>a</i> (%)	43 $\pm$ 7 (4)	39 $\pm$ 2 (4)
Glucose output (μ mol/min per g)	0.45 $\pm$ 0.09 (3)	0.24 $\pm$ 0.06 (5)

tive insensitivity to adrenaline of the increases in glucose output (Ezdinli & Sokal, 1966) and hepatic cyclic AMP concentrations (Exton *et al.*, 1971), and also of studies with inhibitors *in vivo* (Shikama & Ui, 1975*a,b*). However, measurements of glucose output in perfused livers have suggested that the liver can respond to concentrations of about 10 nM (Exton *et al.*, 1971; Saitoh & Ui, 1976; Brungardt *et al.*, 1972) as is confirmed in our experiments; also, 1 nM-isoprenaline can stimulate glucose output (Haylett & Jenkinson, 1972). The activation of phosphorylase by adrenaline is one of the most sensitive hepatic responses to this hormone elicited so far, being at least as sensitive as the increase in glucose output (present work) and more sensitive than the increase

in cyclic AMP, at least in steroid-treated animals (Saitoh & Ui, 1975, 1976).

Activation of glycogen phosphorylase by angiotensin II has not been described previously in an isolated liver preparation. The sensitivity of the rat liver to angiotensin II (minimally effective concentration about 60 pM) resembled that for vasopressin. Also, the sensitivity is of the same order as that exhibited by other tissues to angiotensin II, namely kidney (Barraclough *et al.*, 1967; Laragh & Sealey, 1973) and intestine (Bolton *et al.*, 1975; Regoli & Vane, 1964). Angiotensin II can also activate glycogen phosphorylase in the kidney (Handler & Orloff, 1963).

Relation between phosphorylase activation and glucose output

Hepatic glycogen phosphorylase activity and glucose output are often implicitly taken to be closely related. This is a misapprehension, as there are other fates of glycogen-derived glucose phosphates. Bearing this in mind, the present results may be appraised. Vasopressin and angiotensin II activated glycogen phosphorylase at lower concentrations than those which stimulated glucose output. Also, the extent of phosphorylase activation consistently exceeded that of glucose output. Thus the activity of phosphorylase *a* increased to about 17 μ mol of glucose/min per g of fresh liver, when glucose output was 1–2 μ mol/min per g. It is a reasonable assumption that, in this situation, net glycogen breakdown would exceed glucose output (although direct confirmation was not possible in these short experiments). Therefore in liver from fed animals at least, all three hormones (especially at low concentrations) may cause glycogen degradation to products other than free glucose, e.g. via glycolysis or the pentose phosphate pathway (perhaps for the provision of reducing equivalents or 'energy'). This conclusion is in agreement with the facts that vasopressin causes greater

glycogen breakdown than can be accounted for as released glucose and that concentrations as low as 40–100 pM (which activate phosphorylase) do not stimulate gluconeogenesis (Hems & Whitton, 1973). Also, it accords with the minor or absent stimulation of hepatic gluconeogenesis in livers from fed animals by vasopressin (at least in mouse liver: Ma & Hems, 1975) or adrenaline (Pilkis *et al.*, 1975; Shikama & Ui, 1975b).

The effects of diminution of blood flow in the liver were studied in perfusions where flow was slowed by lowering the input pressure. At flow rates which simulated those produced during hormone-induced vasoconstriction, there was activation of phosphorylase (but not inactivation of synthase), which was about half as extensive as that produced by hormones. In the same livers, however, there was no increase in glucose output. Thus moderate ischaemia appears to cause glycogenolysis (perhaps as a result of hypoxia) for disposal of product glucose phosphates within the liver cell, rather than for release as glucose to blood. Events of carbohydrate metabolism during acute ischaemic oxygen deprivation in liver (Hems & Brosnan, 1970) are not fully understood.

Role of vasoconstriction in hormone effects on the liver

In the perfusions with adrenaline and angiotensin II, vasoconstriction was observed. The perfusions at decreased flow rates showed that this factor could contribute to the activation of phosphorylase but not to any inactivation of synthase. However, for both hormones, ischaemia cannot entirely explain the activation of phosphorylase, which was greater than that produced by ischaemia. Nevertheless, responses of hepatic phosphorylase to administered adrenaline or angiotensin II in intact animals are likely to include direct (as well as indirect) hepatic vasoconstrictor effects, as circulating hormone concentrations are usually high (and uncontrolled) in such experiments.

Vasopressin did not cause vasoconstriction in the perfused liver unless very high concentrations were attained. Therefore the direct hepatic stimulation of glycogen phosphorylase by vasopressin solely involves an effect on liver cells. However, the phosphorylase response in the liver of intact rats (Hems *et al.*, 1975; Keppens & de Wulf, 1975) could still involve a diminution of blood flow, resulting from intestinal vasoconstriction.

Significance of hormone actions on the liver

The importance of hormonal activation of hepatic phosphorylase (which may be presumed to cause increased glycogen breakdown) or glucose output may be ascertained by several criteria: (i) occurrence of hyperglycaemia in intact animals; (ii) attainment

in blood of hormone concentrations that affect the liver; (iii) functional significance of the hepatic actions of hormones.

These criteria can be taken in turn. All the hormones tested here can cause hyperglycaemia *in vivo*. This is well established for adrenaline, where the rise in blood glucose concentration is protracted (Hems *et al.*, 1975), in contrast with the transient hyperglycaemia caused by vasopressin (Hems *et al.*, 1975), which is observed in rats. Adrenaline causes hyperglycaemia in humans, as does vasopressin (Karp *et al.*, 1968). Angiotensin II can cause hyperglycaemia in humans (Ajzen *et al.*, 1968), dogs (Heidenreich & Kook, 1963) and rabbits (Akinkugbe *et al.*, 1967).

Plasma renin activity is increased in patients with diabetic coma (Christlieb *et al.*, 1975), as is the plasma catecholamine concentration (Christensen, 1974). Thus the present results suggest that angiotensin II and adrenaline in blood could contribute to the hyperglycaemia of insulin deficiency. For vasopressin, this possibility has not been studied.

For the three hormones, the minimally effective concentrations which stimulated phosphorylase correspond very closely to those that occur in blood in stressed animals. In haemorrhagic states in particular, concentrations of hormones which would affect the liver (0.1 nM-vasopressin or angiotensin II, 10 nM-adrenaline) have been detected in blood for vasopressin (Ginsburg, 1968), adrenaline (Jakschik *et al.*, 1974) and angiotensin II (Vallotton *et al.*, 1967; Tuck *et al.*, 1975; Scornik & Paladini, 1964; for a review see Laragh & Sealey, 1973). In other situations, concentrations of the same order have been reported, e.g. (1) of adrenaline in response to insulin (Christensen, 1974; Goldfien *et al.*, 1958) or during exercise or hypertension (Chodakowska *et al.*, 1975; Christensen, 1972, 1974; Christensen *et al.*, 1975), (2) of angiotensin II, during starvation (Nocenti *et al.*, 1975) or Na⁺ deprivation (Brown *et al.*, 1972), or hypertension (Boyd & Peart, 1974) and (3) of vasopressin, during stress or dehydration (Ginsburg, 1968).

Adrenaline, vasopressin and angiotensin II are all powerful vasoconstrictor agents, and they all increase in blood during haemorrhagic shock in particular. The liver exhibits sensitivity of the same order as blood vessels to vasopressin (e.g., see Altura, 1970) and angiotensin II (Laragh & Sealey, 1973). Clearly the hepatic glycogenolytic action occurs simultaneously with the pressor actions in the intact animal. The simplest view of these events is that, in shock, hypoxic tissues require glucose for anaerobic glycolysis, which is produced by hepatic glycogenolysis both for consumption within the liver and for release as free glucose. Shock states are associated with hyperglycaemia (Johnson, 1972; Drucker *et al.*, 1974); thus glucose may be required as a fuel in hypoxic tissues, and administered glucose can benefit patients in shock (Drucker *et al.*, 1974). In the intact

animal, events may be very complex, e.g. owing to interrelations among the hormones studied in the present work (Axelrod, 1975; Harrison *et al.*, 1973; Regoli *et al.*, 1974; Uhlich *et al.*, 1975), and to the complexity of hormone effects on splanchnic haemodynamics and metabolism (e.g. Krarup, 1975).

In general, these results add to the mounting body of evidence that hepatic glycogen degradation is stimulated by a wide variety of hormones, in the same manner as is the breakdown of adipose-tissue triacylglycerol.

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