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Stimulation by Vasopressin of Glycogen Breakdown and Gluconeogenesis in the Perfused Rat Liver

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1. Vasopressin (anti-diuretic hormone, [8-arginine]vasopressin) stimulated the breakdown of glycogen in perfused livers of fed rats, at concentrations (50–600 μunits/ml) that have been reported in the blood of intact rats, especially during acute haemorrhagic shock. 2. In perfused livers from starved rats, vasopressin (30–150 μunits/ml) stimulated gluconeogenesis from a mixture of lactate, pyruvate and glycerol. 3. Vasopressin prevented accumulation of liver glycogen in the perfused liver of starved rats, or in starved intact rats. 4. The action of vasopressin on hepatic carbohydrate metabolism thus resembles that of glucagon; the minimum effective circulating concentrations of these hormones are of the same order (100 pg/ml). 5. The stimulation of hepatic glucose output by vasopressin is discussed in connexion with the release of glucose and water from the liver.

The metabolic actions of the hormones of the posterior pituitary gland have not been fully elucidated (for reviews, see Stehle, 1950; Mirsky, 1968). It has long been known that extracts of the neurohypophysis can cause hyperglycaemia (Claude & Baudouin, 1912; Clark, 1928) and prevent the hypoglycaemia due to insulin (Burn, 1923), perhaps as a result of breakdown of liver glycogen (Clark, 1928; Imrie, 1929; for discussion see Stehle, 1950). Since purer preparations of hormones became available vasopressin has been shown to bring about an increase in the concentration of blood glucose in mammals (Bergen *et al.*, 1960; Cash & Kaplan, 1964; Schillinger *et al.*, 1972), possibly through hepatic glycogenolysis (Bergen *et al.*, 1960; Heidenreich *et al.*, 1963). Stimulation of glycogen breakdown by vasopressin and oxytocin in liver slices (Heidenreich *et al.*, 1963) and by oxytocin in the perfused rat liver (Vaisler *et al.*, 1965) has been reported.

The significance of the above effects has been uncertain, since they were generally obtained with concentrations of circulating hormone which were higher than those that occur naturally. The purpose of the present paper is to report stimulation of glycogen breakdown and gluconeogenesis in the perfused rat liver by vasopressin at concentrations that can occur in the intact animal, especially during haemorrhagic shock.

Materials and Methods

Perfusions of rat liver

Male rats of the Sprague–Dawley strain, weighing about 180 g, had free access to a standard diet of rat cubes and to water. Starvation was for 48 h, from about 10:00 h. Rats were anaesthetized with diethyl ether–air, and liver perfusion was carried out with 50 ml of bicarbonate–saline containing albumin and washed rat erythrocytes (Hems *et al.*, 1972). Glucose was added to the perfusions of liver from fed rats so that the initial concentration was 5 mM. In the perfusions of liver from starved rats glucogenic substrates were infused (at 3 ml/h from 15–20 min after the start of perfusion) in a mixture containing 0.5 M-sodium lactate, 0.33 M-glycerol and 0.17 M-sodium pyruvate. Glycogen synthesis was followed during perfusion with 30 mM-glucose plus glucogenic precursors (Hems *et al.*, 1972).

Substrates or vasopressin were infused with a Delta pump (Watson–Marlow Ltd., Falmouth, Cornwall, U.K.). An extract of the neurohypophysis from one rat was prepared in 0.5 ml of 0.1 M-HCl for addition to the perfusion medium: after homogenization the extract was cooled in ice, centrifuged for 20 min at 5000 *g* and the supernatant neutralized with NaOH so that the final volume was 1 ml.

Chemical and analytical methods

Chemicals were of the highest grade commercially available. L-Lactic acid was from Sigma (London) Chemical Co. (Kingston, Surrey, U.K.) and pyruvate from C.F. Boehringer Corp. (London W.5, U.K.). [8-Arginine]vasopressin, which is the natural form of vasopressin in the rat, obtained in solution from Sigma (grade VI: essentially oxytocin-free), was prepared from synthetic vasopressin of activity about 360 units/mg. The amino acid composition of this preparation (after hydrolysis in 6 M-HCl; analysis

kindly performed by Mr. C. Dykes) was that expected of vasopressin; this excludes the possibility of significant contamination with additional peptide material. One unit is defined as the pressor activity of 0.5mg of International Reference Standard extract of neurohypophysis. Pure [8-arginine]vasopressin has an activity of about 400 units/mg. The concentration of vasopressin in each of two batches, checked by assay of its anti-diuretic effect in the ethanol–water-loaded rat (Forsling *et al.*, 1968) was about 80% of the stated activity. This factor was taken into account in calculating the concentration of vasopressin. [8-Lysine]-vasopressin was also from Sigma.

Glucose output from the perfused liver was followed by measuring changes in the concentration of glucose in the perfusion medium. To follow the changes in hepatic glycogen, the two largest liver lobes were removed sequentially (Hems *et al.*, 1972). Glucose was measured by a glucose oxidase method (Krebs *et al.*, 1964). Glycogen was determined as glucose after hydrolysis with fungal glucosidase (Lee and Whelan, 1966). L-Lactate was measured with lactate dehydrogenase (Hohorst, 1963).

Results

Stimulation of glycogen breakdown by vasopressin

During perfusion of livers from fed rats the glucose concentration in the medium attained a steady concentration of 8–12mM. If vasopressin was then added in a single dose to the medium there was a marked efflux of glucose, which slowed after about 40min (Fig. 1). This decline in glucose efflux was not caused by lack of glycogen, as shown by measurements of glycogen at the end of the perfusions. The extent of the increment in glucose concentration was dependent on the initial concentration of vasopressin over the range 50–600 μunits/ml (Fig. 2).

The most likely explanation for the rise in glucose concentration in the medium caused by vasopressin is that it reflected breakdown of liver glycogen. This was confirmed by measuring changes in the hepatic content of glycogen (Fig. 3). The rate of glycogenolysis may be calculated from the change in glycogen content of the two (sequential) liver samples taken in each perfusion, presuming that no large differences in glycogen content exist between the major lobes (Hems *et al.*, 1972). The mean decrease in glycogen content in 60min was 49±14 (3) μmol of glycogen glucose/g in control perfusions and 121±31 (4) in the presence of vasopressin (expressed ±S.E.M. with the number of observations in parentheses). The extra glycogen breakdown caused by vasopressin (about 70μmol of glucose/g) thus amounted to about 400μmol of glucose (since the average liver weight was 5.6g), which was sufficient to account for the extra glucose which appeared in the medium (about

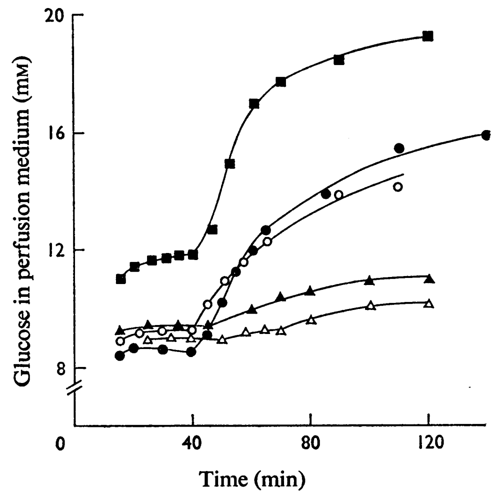

Fig. 1. *Influence of vasopressin on the time-course of glucose output in the perfused liver of fed rats*

Livers were perfused as described in the text. The following additions were made to the perfusion medium after 40min: vasopressin at the following initial concentrations (μunits/ml: 1000, ●; 300, ○; 130, ▲; 5, △), or 0.7ml of an extract of rat neurohypophysis (■). Other details are in the text. Results are from single perfusions.

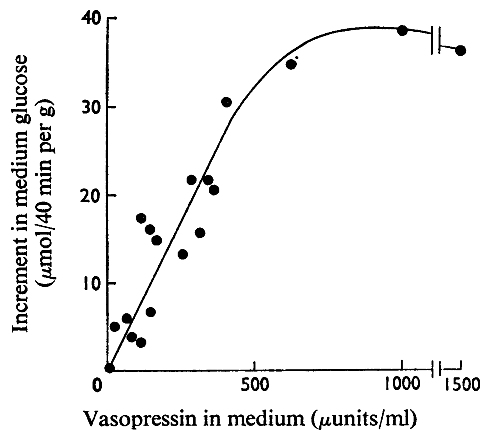

Fig. 2. *Dependence on vasopressin concentration of stimulation of hepatic glucose output*

Livers from fed rats were perfused as described in the text. After 40min vasopressin was added at various initial concentrations. Each point represents the increment in glucose output during the next 40min, determined for each perfusion.

150 μmol). There was no major effect of vasopressin on the concentration of lactate in the perfusion medium (Fig. 3).

The question arises whether a glycogenolytic action of commercial preparations of vasopressin may be regarded as being definitely due to vasopressin. The glycogenolytic effect of the preparation was largely destroyed by incubation in 10mM-thioglycollate (90min, 37°C, vasopressin 100munits/ml) or by a combination of repeated freezing and thawing and standing at room temperature for about 20h. These results (not shown) are compatible with the identification of the glycogenolytic agent as vasopressin, which is inactivated by these procedures. Further, an extract of rat neurohypophysis caused glucose output in the present conditions (Fig. 1). This effect was probably due to vasopressin, since oxytocin (1 munit/ml, in a different experiment) did not bring about marked glucose output (result not shown).

Vasopressin produced no detectable alteration in the rate of flow of perfusion medium (measured by counting the drop rate) in any conditions tested, i.e. at vasopressin concentrations of 5–1000 μunits/ml.

Action of vasopressin on gluconeogenesis

The observed stimulation of glycogen breakdown by vasopressin raised the possibility that the hepatic action of this hormone might resemble that of glucagon, which accelerates both glycogen breakdown and gluconeogenesis (Exton *et al.*, 1970). The action of vasopressin on gluconeogenesis was therefore investigated. In these experiments vasopressin was added to the perfusion medium in five doses at

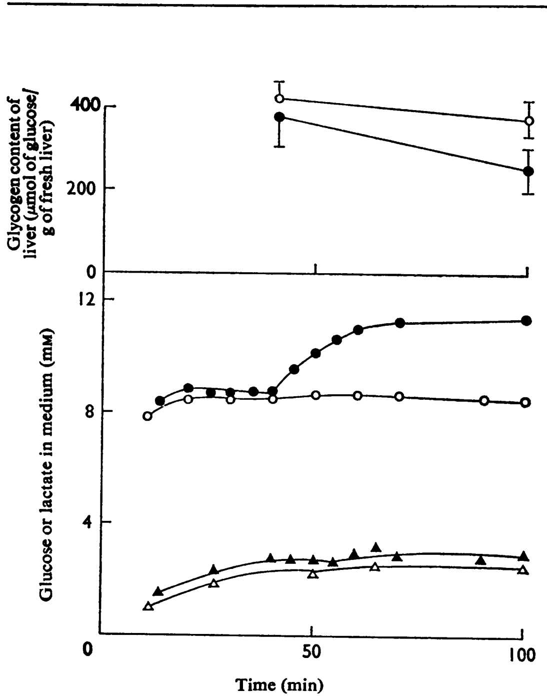

Fig. 3. *Effect of vasopressin on glycogen content of the perfused liver*

Livers from fed rats were perfused as described in the text. After 40min vasopressin was added to an initial concentration of about 700 μunits/ml, and then infused at 200munits/h. An initial liver sample was removed at 41 min. The average liver weight for the remainder of the perfusion was 5.6g. Other details and calculated rates of glycogenolysis are in the text. Results are from three control perfusions (○, △) and four with vasopressin (●, ▲): glucose (○, ●); lactate (△, ▲). Bars indicate the S.E.M.

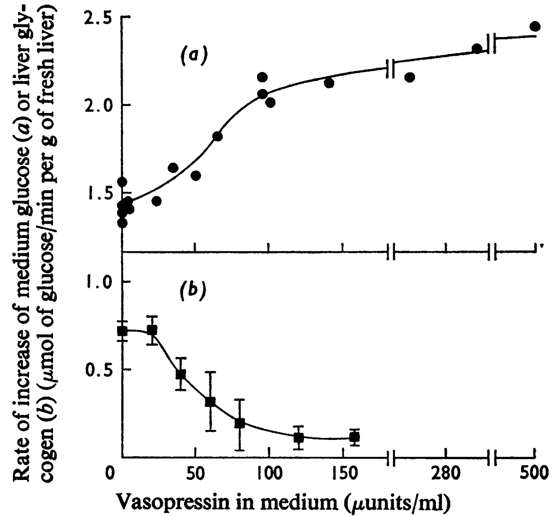

Fig. 4. *Influence of vasopressin on gluconeogenesis or net glycogen accumulation in the perfused liver of starved rats*

Livers were perfused with glucogenic precursors as described in the text. Vasopressin was added at 10 min intervals, from 10 min after the start of perfusion, to the concentration indicated (abscissa) and did not accumulate during perfusion (see the text). Two groups of experiments were carried out: (*a*) measurement of gluconeogenesis (●): rates were measured between 20 and 60 min, and each point represents a single perfusion; the glycogen content of the liver was negligible throughout perfusion; (*b*) measurement of net glycogen synthesis (■): perfusions contained 30mM-glucose in addition to the glucogenic precursors (see Hems *et al.*, 1972) and results are means of three or four measurements (bars indicate S.E.M.).

Table 1. *Effect of vasopressin on net hepatic glycogen synthesis in vivo*

Glucose (1.5M) was infused intravenously (3ml/h) into anaesthetized starved rats and two liver samples were removed sequentially from each rat (Hems *et al.*, 1972). At the time of the second liver sample, the average blood glucose concentration was 33mM. Results are the mean of six experiments ±S.E.M.

Additions to infusion fluid	Glycogen content (μmol of glucose/g)		Calculated rate of net glycogen synthesis (μmol of glucose/min per g)
	After 10min	After 70min	
None	35	72	0.62±0.09
Vasopressin (175munits/ml)	32	44	0.19±0.11

regular intervals, in view of the likelihood that the hormone is rapidly destroyed during perfusion (Little *et al.*, 1966). The concentration of vasopressin in the perfusion medium, measured at the end of the perfusions, was not higher than that produced by each single addition (M. L. Forsling, unpublished work). Hence it is reasonable to consider the vasopressin concentration during each perfusion to be equivalent to that produced by the initial addition of hormone. On this basis, the dependence of gluconeogenesis on vasopressin concentration was evaluated. Gluconeogenesis from an infused mixture of lactate, glycerol and pyruvate was stimulated by vasopressin, in the concentration range 30–150μunits/ml (Fig. 4*a*). In other experiments the effect of vasopressin (800μunits/ml) was tested on gluconeogenesis in the absence of added precursors; no major stimulation occurred (results not shown).

Inhibition of glycogen accumulation in the perfused liver by vasopressin

In the perfused liver from starved rats, maximal net glycogen accumulation requires the participation of circulating glucogenic precursors as carbon sources, and of glucose to direct newly formed hexose phosphates into glycogen (Hems *et al.*, 1972). Since vasopressin stimulated gluconeogenesis and also glycogen breakdown it was decided to determine its action in this situation (Fig. 4*b*). Glycogen accumulation was inhibited by vasopressin and the dependence of this effect on vasopressin concentration (Fig. 4*b*: evaluated as described above) closely resembled that of its action (in separate perfusions), on gluconeogenesis (compare Figs. 4*b* and 4*a*). In these experiments, which differed from those shown in Fig. 4*a* in that 30mM-glucose was present, there were no clear-cut effects of vasopressin on the total synthesis of glucose (i.e. the sum of the changes in liver glycogen plus blood glucose). In other experiments, [8-lysine]-vasopressin was added to perfusions and appeared to inhibit glycogen synthesis over about the same concentration range as [8-arginine]-vasopressin (results not shown).

Action of vasopressin on net glycogen accumulation in vivo

To confirm that vasopressin can affect glycogen metabolism *in vivo* its action was investigated in the intact rat. In fed rats it may be difficult to follow changes in the concentration of liver glycogen. Therefore the effect of vasopressin *in vivo* was studied in starved rats, anaesthetized with Nembutal, during intravenous infusion of glucose (Hems *et al.*, 1972). Vasopressin inhibited net glycogen synthesis in this situation (Table 1).

Discussion

The above experiments show that vasopressin can stimulate the breakdown of glycogen in the liver. This confirms the results obtained with higher concentrations of vasopressin in slices (Heidenreich *et al.*, 1963), and also the inference that was drawn from experiments *in vivo* in which vasopressin was injected into the hepatic portal vein (Bergen *et al.*, 1960). The effects of vasopressin in the perfused rat liver were obtained with concentrations in the range 30–600μunits/ml. Such concentrations can occur in blood in the intact rat, especially during acute haemorrhage (Ginsburg & Heller, 1953; Forsling *et al.*, 1971) and during 'shock' or 'stress' of other types (see Ginsburg, 1968). Hence stimulation by vasopressin of the breakdown of liver glycogen may be of importance *in vivo*, at least in the rat. Inhibition of net glycogen accumulation was observed during intravenous infusion of vasopressin, confirming that vasopressin can affect hepatic glycogen metabolism in the intact animal. The prevention of glycogen accumulation in starved rats, *in vivo* or in the perfused liver, is likely to be due to the same action of vasopressin as the stimulation of glycogen breakdown in fed rats.

The sensitivity of the liver to vasopressin was of the same order as the sensitivity observed for the pressor action (see Dekanski, 1952), which is exerted on extrahepatic tissues. Hence the action of vasopressin on the liver represents a third main function of this

hormone in the rat, in addition to its anti-diuretic and pressor effects. The pressor and hepatic actions are similar in their requirement for relatively higher concentrations of the hormone, as can occur during 'shock'. A role of vasopressin in the control of hepatic metabolism during stress would exhibit similarities to that proposed for adrenaline. In unstressed animals the concentrations in blood of vasopressin and adrenaline may be too low to affect the liver.

The significance of the hepatic action of vasopressin may be at least twofold. States of 'shock' are usually associated with hyperglycaemia (see Johnson, 1972). The present results suggest that vasopressin may contribute to the accelerated release of hepatic glucose during 'shock'. Secondly, the action of vasopressin may be important in regard to the water that is stored in conjunction with glycogen, and can amount to 2–4 times the weight of glycogen (Fenn, 1939). This water can be mobilized, e.g. during perfusion (Mortimore, 1961) or starvation (Herrera & Freinkel, 1968). In a fed rat of about 200g the hepatic glycogen content may be as high as 1g and the associated water up to 3ml. This store of hepatic water could amount to as much as one-third of the plasma volume, which may be mobilized rapidly without prejudice to organ function. This would be in accord with the accepted role of vasopressin in the preservation of plasma volume, composition and pressure.

Since vasopressin caused glycogen breakdown, and also moderate stimulation of gluconeogenesis (in livers from starved rats that were perfused in the absence of initial glucose), its actions on the liver resemble those of glucagon (Exton *et al.*, 1970). The minimum effective circulating concentrations of these hormones (in the rat) are of the same order, i.e. about 100pg/ml, as 1 μunit of vasopressin corresponds to 2.5pg (for discussion of hepatic sensitivity to glucagon see Sokal, 1966; Exton *et al.*, 1970). As with glucagon (Pauk & Reddy, 1971), the liver of the starved rat appeared to be more sensitive to vasopressin than the liver of the fed rat.

In the kidney, vasopressin can stimulate glycogen breakdown (Darnton, 1967) and gluconeogenesis (Stumpf *et al.*, 1972). Since both the renal action of vasopressin (Beck *et al.*, 1971) and the hepatic actions of glucagon and adrenaline (Exton *et al.*, 1970) may be mediated by an increase in the tissue concentration of 3′:5′-cyclic AMP, it is possible that the action of vasopressin on liver carbohydrate metabolism may be similarly mediated. This remains to be elucidated, as does the full significance of the hormones of the posterior pituitary gland in metabolic events.

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