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Inhibition of Fatty Acid Synthesis and Stimulation of Glycogen Breakdown by Vasopressin in the Perfused Mouse Liver

By GARY Y. MA and DOUGLAS A. HEMS
Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, U.K.

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1. Vasopressin (anti-diuretic hormone, [8-arginine]vasopressin) inhibited the synthesis *de novo* of fatty acids (measured with ^3H_2O and U-^{14}C-labelled lactate or U-^{14}C-labelled glucose) and stimulated glycogen breakdown in the perfused liver of fed mice. 2. The concentration dependence of these effects (range 200–1000 μunits/ml, i.e. 0.5–2.5 ng/ml) resembled that for the action on glycogen breakdown which was previously reported for rat liver. 3. The appearance of newly synthesized fatty acids in both phospholipids and triglycerides was inhibited by vasopressin, whereas synthesis of cholesterol was less affected. 4. Inhibition of hepatic lipogenesis by vasopressin is the most potent short-term hormonal action on this process yet reported. Aspects of the effect are discussed, including the lack of a role for cyclic AMP, and a possible link with vasopressin action on glycogen metabolism.

Vasopressin (anti-diuretic hormone) stimulates glycogen breakdown in the rat liver, as has been established in perfusion experiments (Hems & Whitton, 1973; Kirk & Hems, 1974). This effect occurs at concentrations (0.1–2 ng/ml; Hems & Whitton, 1973) which can occur in the intact rat, e.g., in haemorrhagic shock (see Ginsburg, 1968, for references). Further, the hepatic action of vasopressin is manifest in the absence of any associated decrease in the rate of blood flow and is therefore not due to overall hypoxia resulting from a hepatic pressor effect. The hepatic action of vasopressin constitutes a third major systemic action of vasopressin (at least in rats) in addition to the renal and pressor effects and deserves further study.

The question arises as to whether vasopressin exerts any other direct hepatic actions on metabolic processes. In the present paper inhibition of fatty acid synthesis by vasopressin in the perfused liver of the mouse is described for a concentration range which also stimulated glycogen breakdown and which resembled that previously reported for the action of vasopressin in stimulating glycogen breakdown in the rat liver (Hems & Whitton, 1973).

Materials and Methods

Chemicals and animals

Fed female mice, aged about 10 weeks, were bred in the Department of Biochemistry at Imperial College (Salmon *et al.*, 1974). Sources of chemicals have been given previously (Elliott *et al.*, 1971; Salmon & Hems, 1973). Batches of vasopressin were checked for activity by bioassays which were again kindly performed by Dr. Mary Forsling (Department of Physiology, Middlesex Hospital; see Hems & Whitton, 1973).

Perfusion of mouse liver

Livers were perfused with bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing albumin and aged human erythrocytes, as previously described (Salmon *et al.*, 1974), with minor modifications. During 'pre-perfusion', warmed bicarbonate-buffered saline, gassed with O_2+CO_2 (95:5) and containing 20mM-glucose, was used to sustain glycogen content. In one series of perfusions, 60ml of medium (containing 15mM-glucose) was recirculated (as usual), and fatty acid or cholesterol synthesis was followed by the incorporation of ^3H from ^3H_2O and ^{14}C from [U-^{14}C]lactate (initially about 10mM) as previously described (Salmon *et al.*, 1974); radioactive precursors were added after 60min and vasopressin (4munits/ml, in one group) after 45min (and subsequently at 15min intervals). Livers were analysed after 3h. In a second series, medium (120ml) containing glucose (15mM) and lactate (10mM) was recirculated for 30min, when radioisotopes (^3H_2O and [U-^{14}C]glucose) and, in one group, vasopressin were added; from this point on, the medium was no longer recirculated, and perfusion continued for 45–70min until input medium ran out. Livers were then analysed. In this series of perfusions glucose in the perfusate was measured after 30min and in the effluent medium at 5min, 15min and 30min after hormone addition.

In all groups vasopressin (0.2–4munits/ml) did not cause any decrease in overall blood flow rate when measured by drop-counting.

Analytical methods

Total rates of lipid synthesis (from all sources of acetyl units) were calculated from ^3H contents of lipids (Salmon *et al.*, 1974), by using the quotient (^3H in lipid, expressed in d.p.m.)/(specific radioactivity of perfusate ^3H_2O, expressed as d.p.m. per μg-atom of total water H). From this value, amounts of newly synthesized lipid, expressed in C_2 units, may be obtained for fatty acids by dividing by 1.7 and for cholesterol by dividing by 1.4 (Windmueller & Spaeth, 1966, 1967; Brunengraber *et al.*, 1973). This method of expressing results involves no presumptions about the nature of fatty acid products, in contrast with the use of a factor such as 13.3 to calculate molar rates of fatty acid synthesis (e.g. Salmon *et al.*, 1974; Hems *et al.*, 1975*a*). The above factors (1.7 and 1.4) largely reflect an 'isotope effect' which involves discrimination against ^3H (compared with ^1H) by enzymes, by factors of about 2.3 for fatty acids and 2.8 for cholesterol (Windmueller & Spaeth, 1966, 1967). Therefore calculated rates of lipid synthesis are minimum estimates, as the presumption is made that ^3H penetrates all relevant pools of ^1H (in water or lipid precursors) within a short period. This presumption is supported by the observation that product fatty acids in perfused livers can be labelled (in a few hours) with ^2H_2O throughout the molecule (Wadke *et al.*, 1973). The validity of these calculations has been discussed (Salmon *et al.*, 1974; Brunengraber *et al.*, 1972, 1973; Thurman & Scholz, 1973; Wadke *et al.*, 1973; Clark *et al.*, 1974). So far, we consider that the use of ^3H_2O to measure total rates of lipogenesis is acceptable, especially if an excess of reducing substrates (e.g. lactate) is avoided (see Clark *et al.*, 1974) and also if findings can be supported with the use of ^{14}C-labelled precursors.

Glycogen, glucose and lactate were measured as previously described, by using enzyme-based procedures (Elliott *et al.*, 1971).

Lipids were extracted and separated as previously described (Salmon & Hems, 1973). After saponification of the washed lipid extract, or of major lipid classes separated by t.l.c., fatty acids were extracted in light petroleum (b.p. 40–60°C), and cholesterol was isolated as digitonide (Brunengraber *et al.*, 1973), for determination of ^3H and ^{14}C contents (simultaneously) by liquid-scintillation spectrometry (Salmon *et al.*, 1974). The ^{14}C in glucose was similarly determined after separation (Elliott *et al.*, 1971).

Results

Effect of vasopressin at different concentrations on fatty acid synthesis and glycogen breakdown

The effect of vasopressin at different concentrations was assessed in non-recirculating perfusions, so that the hormone concentration would not change during perfusion. Over the concentration range 200–1000 μunits/ml (0.5–2.5 ng/ml), vasopressin inhibited the synthesis *de novo* of fatty acids from all sources (measured with ^3H_2O), and from [^{14}C]glucose (Fig. 1). In the same perfusions vasopressin stimulated the output of glucose and the breakdown of glycogen (Fig. 2). After hormone administration the glucose concentration in effluent blood increased by 0.5–3.0 mM within 5 min and then remained constant; the amount of glucose released was dependent on vasopressin concentration, as was glycogen breakdown (Fig. 2).

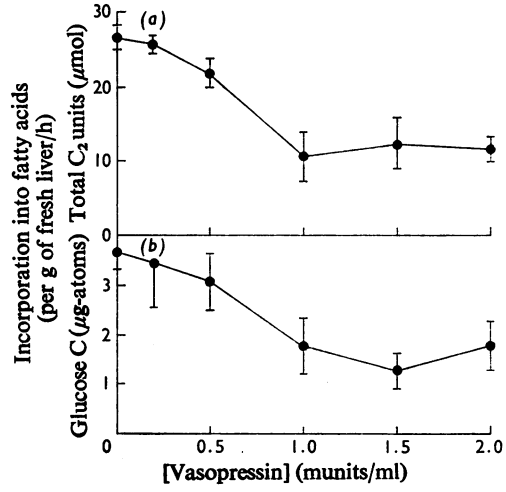

Fig. 1. *Effect of vasopressin at different concentrations on fatty acid synthesis*

Livers were perfused with non-recirculating medium. Fatty acid synthesis was followed with (*a*) ^3H_2O (total rate) and (*b*) [^{14}C]glucose, in the presence of various concentrations of vasopressin. For other details, see the text. Results are means of three or four perfusions, and bars indicate S.E.M.

Effect of vasopressin on glyceride and cholesterol synthesis

In a separate series of experiments, the synthesis of lipids was followed in perfusions where the medium was recirculated and contained [^{14}C]lactate, a favoured precursor for fatty acid synthesis in the liver (Salmon *et al.*, 1974). The synthesis of fatty acid (total rate and that from lactate) was inhibited by vasopressin (Table 1), whereas cholesterol synthesis (total rate) was less affected (Table 1). Incorporation of lactate C into cholesterol was inhibited, but that into glucose was not altered by vasopressin (Table 1); the lactate concentration in the perfusion medium decreased by about 1 mM (from 11–12 mM initially) in these perfusions, and vasopressin did not affect this change (results not shown). Thus the extra glucose

output (about 50 μmol; results not shown) was largely a result of glycogen breakdown (rather than gluconeogenesis). The percentage of ^{14}C or ^{3}H in phospholipid fatty acid was about 45% (of the total in fatty acid) in control perfusions and about 35% in perfusions with vasopressin (results not shown). Corresponding values for triglyceride fatty acid were 45 and 60% respectively. Thus incorporation of ^{3}H or ^{14}C into all major species of hepatic glycerides was inhibited by vasopressin, as is revealed by multiplying these values for the distribution of radioactivity by the results in Table 1.

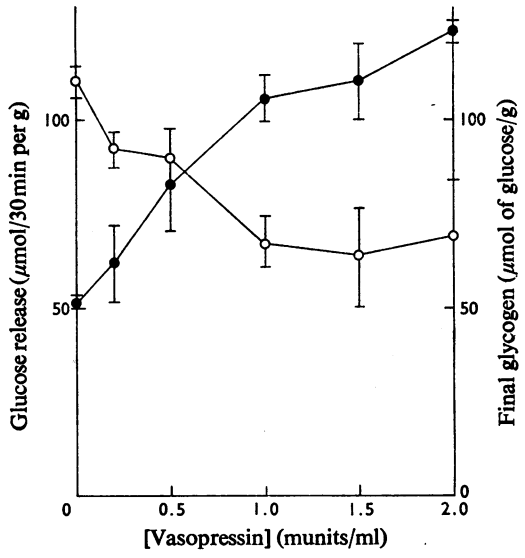

Fig. 2. *Effect of vasopressin at different concentrations on glucose output and glycogen breakdown*

Livers were perfused with non-recirculating medium. Glucose output (●) over 30min was calculated from the difference in concentration between input and effluent medium; the latter reached a steady value within 5min of vasopressin addition and remained steady for at least 30min. Glycogen (○) was measured at the end of the perfusion. For other details, see the text. Results are from the same perfusions as in Fig. 1.

Discussion

These experiments, performed under conditions optimal for hepatic lipogenesis (Salmon *et al.*, 1974), show that vasopressin can inhibit fatty acid synthesis in the mouse liver over the same concentration range as can stimulate glycogen breakdown in the liver of the mouse (the present study) or rat (Hems & Whitton, 1973; Kirk & Hems, 1974). This result confirms the significance of the hepatic action of vasopressin, which has now been demonstrated in two rodent genera at concentrations that can occur in the intact animal (Ginsburg, 1968).

The inhibitory action of vasopressin on lipogenesis (minimally effective dose about 500pg/ml) is the most potent such effect yet reported for any active principle (hormonal or otherwise). Glucagon, for example, when tested in the perfused liver, does not appear to produce short-term inhibition of fatty acid synthesis, even at high concentrations (Raskin *et al.*, 1974).

The route of entry of ^{3}H from $^{3}H_2O$ into fatty acids is complex, and care is required in the use of this technique to calculate molar rates of fatty acid synthesis [e.g. see the Materials and Methods section of the present paper and the Discussions of Clark *et al.* (1974) and Thurman & Scholz (1973)]. In the procedure used in the present paper, the 'isotope effect' (i.e. discrimination in cells between ^{3}H and ^{1}H) is taken into account, but ^{3}H and ^{1}H are otherwise presumed to mix extensively (in metabolites as well as in water) before entry into fatty acid (Windmueller & Spaeth, 1966; Brunengraber *et al.*, 1972, 1973; Wadke *et al.*, 1973); hence the calculated rates of synthesis are minimum estimates. It is possible that a hormone (e.g. vasopressin) might alter the rate of entry of ^{3}H into fatty acids through a mechanism unrelated to the conversion of lactate C, glucose C or C_2 units into fatty acids. In this event the calculated rates of fatty acid synthesis and estimates of proportional changes in lipogenesis

Table 1. *Influence of vasopressin on synthesis of lipids and glucose*

Livers were perfused with recirculating medium containing $^{3}H_2O$, [U-^{14}C]lactate (initially about 12mM) and glucose (initially about 15mM). Radioisotope-labelled water and lactate were added after 60min. In one group, vasopressin (4munits/ml) was added after 45min and then every 15min. Livers and perfusate were analysed after 3h. For further details, see the text. Results are means±S.E.M. of four control perfusions and six with vasopressin. *$P<0.002$ (against appropriate control).

	Total lipid synthesized (μmol of C_2 units/2h per g of fresh liver)		Lactate C incorporated (μg-atoms/2h per g)		
	Fatty acid	Cholesterol	Fatty acid	Cholesterol	Perfusate glucose
Control	79.7±2.9	3.7±0.3	76.1±13.6	1.46±0.27	241±20
Vasopressin	37.3±3.9*	3.1±0.6	20.7±3.7*	0.54±0.06*	243±19

(based on the factors described in the Materials and Methods section) would be wrong. However, the present observation that vasopressin inhibits synthesis of fatty acids from lactate C and from glucose C establishes that the flow of C from simple precursors to glyceride fatty acid is inhibited by vasopressin.

In the present conditions the major sources of C_2 units for the synthesis of fatty acids are glycogen and lactate (Salmon *et al.*, 1974). The inhibitory action of vasopressin on lipogenesis involves both these sources (as incorporations of ^3H and lactate C were inhibited to similar extents) and may therefore include actions on glycogen metabolism, and action at site(s) between pyruvate and product. The dependence of the effects on glycogen metabolism and lipogenesis on vasopressin concentration was identical. This is in marked contrast with glucagon, which stimulates glycogen breakdown at concentrations over the range 0.1–1 nM (Exton *et al.*, 1971), but apparently exerts no parallel effect on fatty acid synthesis (Raskin *et al.*, 1974). Hence the inhibitory action of vasopressin on lipogenesis is probably linked with the stimulation of glycogen breakdown. A simple explanation for this link would be that the action of vasopressin on both processes has features in common. Such a link cannot involve a direct effect of vasopressin on provision of C_2 residues from glycogen (since this was increased); rather, there is redirection of glycogen-derived C away from lipogenesis, and towards, e.g., glucose release. Of course the glucose release induced by vasopressin was greater than could be accounted for by inhibition of fatty acid synthesis and was mainly due to extra glycogen breakdown; thus the difference between final glycogen contents in these groups of perfusions (about 80 μmol/liver) was of the same order as the extra glucose release (about 90 μmol) due to vasopressin. Such an inverse association between rates of glycogen breakdown and lipogenesis (from sources other than glycogen) is reminiscent of the observed proportionality between initial glycogen content and fatty acid synthesis from glucose in the perfused mouse liver (Salmon *et al.*, 1974). It is unlikely that the action of vasopressin on lipogenesis is simply due to prior depletion of glycogen, as the effect was so marked in relatively short perfusions and since conversion of lactate into lipid was at least equally decreased.

Vasopressin inhibited the synthesis from lactate C of all lipids measured. Thus the action of vasopressin on lipogenesis must be ultimately located at a site which is regulatory in the synthesis from lactate of both fatty acids and cholesterol. This could, for example, involve the step catalysed by pyruvate dehydrogenase, or multiple sites.

The question arises of the mechanism of action of vasopressin on the liver. One issue concerns the possible role of vasoconstriction and hypoxia. However, the metabolic effects of vasopressin in the perfused liver of the rat (Hems & Whitton, 1973) or mouse (the present study) can occur in the absence of any change in blood flow rate; vasoconstriction in these preparations does not occur until concentrations of 10 munits/ml or more are attained (P. D. Whitton, C. J. Kirk & D. A. Hems, unpublished work). Therefore simple hypoxia due to overall hepatic vasoconstriction does not underlie the metabolic effect of vasopressin, at least at low concentrations such as those tested here.

Vasopressin can activate glycogen phosphorylase *in vivo* (Keppens & de Wulf, 1975; Hems *et al.*, 1975*b*). In rats at least, this does not involve 3′:5′-cyclic AMP (Kirk & Hems, 1974) or protein kinase activation (Keppens & de Wulf, 1975). The mechanism of action of vasopressin on glycogen metabolism and lipid synthesis still has to be clarified.

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