Interactions between Vasopressin and Glucagon on Ketogenesis and Oleate Metabolism in Isolated Hepatocytes from Fed Rats

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Vasopressin (10nM) inhibited ketogenesis (56%) in hepatocytes from fed rats when oleate (1mM) was the substrate, but had no effect with butyrate (10mM). The hormone increased the accumulation of lactate and stimulated the esterification of [$1\text{-}^{14}\text{C}$]oleate (70%). These effects of vasopressin were reversed by glucagon (10nM). The physiological implications of these findings are discussed.

Vasopressin and glucagon exert a number of rapid effects on the liver, including stimulation of glycogenolysis (Exton *et al.*, 1972; Hems *et al.*, 1978b), activation of phosphorylase (Hems *et al.*, 1975; Keppens & De Wulf, 1975), enhancement of gluconeogenesis (Exton & Park, 1968; Whitton *et al.*, 1978) and inhibition of lipogenesis (Ma & Hems, 1975; Cook *et al.*, 1977). Their mode of action, however, is likely to be different, because, unlike glucagon, vasopressin does not increase the hepatic content of cyclic AMP (Hems *et al.*, 1978a) and may act via alterations of Ca^{2+} fluxes in cells (for review see Hems, 1979). It has been reported that vasopressin activates pyruvate dehydrogenase (Hems *et al.*, 1978c) and phosphatidylinositol turnover (Kirk *et al.*, 1979) in rat liver. It is well established that glucagon (or cyclic AMP) stimulates ketogenesis (Heimberg *et al.*, 1969), and one postulated mechanism (McGarry *et al.*, 1978a) for this effect is inhibition of lipogenesis and concomitant lowering of [malonyl-CoA], an inhibitor of entry of long-chain fatty acyl-CoA into the mitochondria for oxidation (McGarry *et al.*, 1978b). More recent evidence, however, suggests that glucagon (or cyclic AMP) may also inhibit the esterification of long-chain acyl-CoA (Klausner *et al.*, 1978; Benito *et al.*, 1979).

The present paper reports a comparison of the effects of vasopressin and glucagon on ketogenesis and oleate metabolism in hepatocytes isolated from fed rats. A novel finding is that vasopressin inhibits ketogenesis when oleate is the added substrate and stimulates esterification of [$1\text{-}^{14}\text{C}$]oleate; glucagon reverses these effects of vasopressin.

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Experimental

Female rats (180–250g) of the Wistar strain were fed *ad libitum* on Oxoid breeding diet for rats and mice (Oxoid, London S.E.1, U.K.). They were anaesthetized with Nembutal (60mg/kg body wt.; solution in 0.9% NaCl).

All enzymes and coenzymes were obtained from Boehringer Corp. (London), London W.5, U.K. [Arginine]vasopressin (grade VIII) and glucagon were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. [$1\text{-}^{14}\text{C}$]Oleate and $^3\text{H}_2\text{O}$ were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Isolated hepatocytes were prepared essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974). Preparation of hepatocytes was commenced between 9:30 and 10:30 h.

The incubation procedure and measurements of esterification and removal of [$1\text{-}^{14}\text{C}$]oleate were as described by Whitelaw & Williamson (1977). Lipogenesis was measured with $^3\text{H}_2\text{O}$ by the method described by Harris (1975). The following metabolites were determined in the neutralized HClO_4 extracts by enzymic methods: glucose (Slein, 1963), L-lactate (Hohorst *et al.*, 1959), acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962).

The metabolite changes between 20 and 60 min were calculated from plots of the values at 20, 40 and 60 min and the results are expressed as $\mu\text{mol/min per g fresh wt. of cells}$, $\pm\text{s.d.}$, with the numbers of observations in parentheses.

Results and Discussion

Effects on ketogenesis from oleate and butyrate

Glucagon (10nM) increased the rate of ketogenesis from oleate by 64% (Table 1), which is

Table 1. Comparison of effects of vasopressin and glucagon on ketogenesis, and glucose and lactate production, in hepatocytes from fed rats

For details see the Experimental section. The final concentrations of the substrates and hormones are given in the Table. Results are mean values $\pm$ S.D., with the numbers of experiments in parentheses. Production of ketone bodies, glucose and lactate is expressed as $\mu\text{mol}/\text{min}$ per g wet wt. of cells. The values for cells incubated in the absence of substrate were: ketone bodies, 0.03 ± 0.013 (4); glucose, 0.71 ± 0.13 (4); lactate, 0.82 ± 0.12 (4). Values that are significantly different from those without addition of hormone are indicated by: $^{**}P < 0.005$.

Additions	Oleate (1 mM)			Butyrate (10 mM)
	Ketone bodies	Glucose	Lactate	Ketone bodies
None	0.45 ± 0.13 (11)	0.95 ± 0.15 (12)	0.0	1.25 ± 0.18 (3)
Glucagon (10 nM)	0.74 ± 0.12 (10)**	1.86 ± 0.72 (9)**	0.0	1.20 ± 0.14 (3)
Vasopressin (10 nM)	0.20 ± 0.085 (12)**	1.95 ± 0.47 (12)**	0.56 ± 0.27 (8)	1.25 ± 0.18 (3)
Glucagon (10 nM) + vasopressin (10 nM)	0.52 ± 0.10 (8)	2.19 ± 0.75 (8)**	0.0	1.25 ± 0.23 (3)

similar to the increase observed with dibutyl cyclic AMP (0.01 mM) in hepatocytes from weanling rats (Benito *et al.*, 1979). In marked contrast, vasopressin (10 nM) decreased ketogenesis from oleate by 56% (Table 1), and this was accompanied by a decrease in the ratio of the rate of production of 3-hydroxybutyrate/acetoacetate from 0.91 ± 0.49 (9) to 0.54 ± 0.31 (9) ($P < 0.05$). This change in ratio suggests a more oxidized mitochondrial free NAD^+/NADH system owing to a decrease in oleate oxidation. When both hormones were present at equimolar concentrations (10 nM), the rate of ketogenesis from oleate was not significantly different from the control without addition of hormone. Glucagon did not alter the rate of ketogenesis when butyrate was the substrate, which is in agreement with the finding that cyclic AMP has no effect on ketogenesis from this substrate (Whitelaw & Williamson, 1977). Vasopressin also caused no significant change in the rate of ketogenesis from butyrate (Table 1). Vasopressin (10 nM) had no effect on the rate of ketogenesis from oleate in hepatocytes from starved (24 h) rats [control (4), $1.77 \pm 0.21 \mu\text{mol}/\text{min}$ per g fresh wt. of cells; plus vasopressin (4), 1.64 ± 0.41]. Siess *et al.* (1978) found no effect of vasopressin on the endogenous rate of ketogenesis in starved hepatocytes.

Glucose and lactate production

Addition of vasopressin or glucagon caused a similar increase (about 100%) in glucose release to the medium (Table 1); this was presumably due to the activation of glycogenolysis. Oleate (1 mM) decreased the accumulation of lactate to virtually zero, and the addition of glucagon resulted, in some experiments, in the disappearance of a portion of the lactate originally present. In marked contrast, addition of vasopressin gave a rate of lactate production which was approx. 70% of that in the absence of oleate (Table 1). Hems *et al.* (1978b)

found no effect of vasopressin on lactate production by hepatocytes from fed rats in the absence of added fatty acid, whereas addition of glucagon resulted in inhibition of lactate formation.

Removal and esterification of [$1\text{-}^{14}\text{C}$]oleate

The absence of an effect of vasopressin on ketogenesis from butyrate, and the inhibition of ketogenesis when oleate was the added substrate, suggested that the hormone was either decreasing oleate removal or stimulating its esterification. To decide between these possibilities, the effect of vasopressin on [$1\text{-}^{14}\text{C}$]oleate metabolism was studied. [$1\text{-}^{14}\text{C}$]Oleate removal was slightly (15%; $P < 0.05$) stimulated by vasopressin, whereas glucagon had no effect (Table 2). Esterification of [$1\text{-}^{14}\text{C}$]oleate was stimulated (70%) by vasopressin. Glucagon alone decreased the rate of esterification, though not significantly, and when added with vasopressin the rate of esterification was similar to that of the control with oleate alone (Table 2). Thus a major effect of vasopressin under these conditions is to increase the conversion of oleoyl-CoA into esterified products, and therefore presumably less is oxidized to acetyl-CoA. Vasopressin had no effect on esterification of [$1\text{-}^{14}\text{C}$]oleate in hepatocytes from starved (24 h) rats (results of two experiments: controls, 0.11, $0.085 \mu\text{mol}$ of [$1\text{-}^{14}\text{C}$]oleate esterified/min per g fresh wt. of cells; plus vasopressin, 0.12, 0.083).

Possible mechanisms and physiological implications

If it is accepted that vasopressin decreases ketogenesis from oleate by increasing its conversion into triacylglycerol, this raises the question of the possible mechanisms. In hepatocytes from fed rats, vasopressin increases glycolytic flux (Table 1), activates pyruvate dehydrogenase (Hems *et al.*, 1978c) and therefore might be expected to stimulate lipogenesis and increase [malonyl-CoA]. The latter

Table 2. Comparison of effects of vasopressin and glucagon on [$1\text{-}^{14}\text{C}$]oleate removal and esterification in hepatocytes from fed rats

For details see the Experimental section. [$1\text{-}^{14}\text{C}$]Oleate (1 mM) was present in all experiments, and the final concentrations of the hormones are given in the Table. The results are mean values $\pm$ S.D., with the numbers of observations in parentheses. Oleate removal or esterification is expressed as $\mu\text{mol}/\text{min}$ per g wet wt. of cells. Values that are significantly different from those for oleate alone are indicated by: * $P < 0.05$; ** $P < 0.005$.

Additions	Measurement		Percentage esterified
	Removal of [$1\text{-}^{14}\text{C}$]oleate	Esterification of [$1\text{-}^{14}\text{C}$]oleate	
None	0.33 ± 0.044 (8)	0.17 ± 0.050 (7)	51
Glucagon (10 nM)	0.31 ± 0.032 (6)	0.13 ± 0.033 (5)	42
Vasopressin (10 nM)	0.38 ± 0.073 (8)*	0.29 ± 0.066 (7)**	76
Glucagon (10 nM) + vasopressin (10 nM)	0.32 ± 0.084 (6)	0.18 ± 0.048 (5)	56

inhibits the entry of long-chain acyl-CoA into the mitochondria for oxidation (McGarry *et al.*, 1978a), and thus ketogenesis would be decreased. However, in perfused mouse liver vasopressin has been reported to inhibit lipogenesis (Ma & Hems, 1975). We found inhibition (30%; $P < 0.01$) of $^3\text{H}_2\text{O}$ incorporation into lipid by vasopressin (10 nM) with hepatocytes from fed rats when oleate was absent [controls (3), $0.14 \pm 0.02 \mu\text{mol}$ of $^3\text{H}_2\text{O}$ incorporated/min per g fresh wt. of cells; plus vasopressin (3), 0.10 ± 0.01], but the decrease in the rate of lipogenesis was not significant when oleate was present [controls (3), 0.11 ± 0.02 ; plus vasopressin (3), 0.089 ± 0.02]. Kirk & Hems (1979) found that vasopressin did not significantly decrease lipogenesis in perfused rat liver when glucose and lactate were the substrates. In the context of the present experiments, the important point to stress is that there is no evidence for an increased rate of lipogenesis in the presence of vasopressin. Alternatively, the increased glycolytic flux in the presence of vasopressin might increase the availability of glycerol 3-phosphate for the esterification of oleate. Although the antiketogenic action of vasopressin appears to be related to lactate production (Table 1), it is possible that vasopressin might activate glycerol phosphate acyltransferase (EC 2.3.1.15). Recent experiments have shown that the hepatic mitochondrial activity of this enzyme is responsive to altered hormonal status; insulin increases the activity *in vitro* (Bates *et al.*, 1977) and diabetes or anti-insulin serum decreases it *in vivo* (Bates & Saggerson, 1979). The antagonistic effects of glucagon in the present experiments may be due to the decreased glycolytic flux (Table 1; Hems *et al.*, 1978b), which in turn may explain the decreased [glycerol 3-phosphate] observed in hepatocytes from fed rats when glucagon was present (Bremer *et al.*, 1978).

The blood concentrations of vasopressin increase in a number of stress situations, including haemorrhage (Errington & Rocha E Silva, 1972) and acute

insulin-deficient diabetic coma (Walsh *et al.*, 1979). The first 24 h after human injury is often associated with hyperglycaemia and hyperlactataemia, whereas blood ketone bodies are within the normal range (Wedge *et al.*, 1976). The present experiments provide a possible explanation for these findings in the human: an increase in circulating vasopressin would be expected to promote hepatic glycogenolysis and glycolysis and decrease ketogenesis, despite the increased flux of non-esterified fatty acids to the liver owing to adrenergic stimulation of adipose-tissue lipolysis. This action of vasopressin would seem advantageous in the 'stressed' fed state, because increased availability of ketone bodies for the brain would not be required in view of the prevailing hyperglycaemia. The failure of raised vasopressin concentrations to have a significant effect on ketogenesis in diabetic coma is presumably in part due to the fact that the liver is in a gluconeogenic mode in this situation and plasma glucagon is greatly increased.

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