Metabolic effects of vasopressin infusion in the starved rat

Reversal of ketonaemia

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1. The effects of vasopressin on the metabolism of starved rats were investigated by using a constant-infusion regimen (50 pmol/kg body wt. per min, after an initial loading dose of 150 pmol/kg body wt.). 2. Blood ketone bodies decreased by 50% in 10 min, and this was accompanied by a 60% decrease in the plasma non-esterified fatty acids. 3. Blood glucose increased by 0.9 mM within 5 min and decreased to control values over the 40 min infusion. Small increases in lactate and pyruvate also occurred. 4. Plasma insulin was not increased by vasopressin infusion. 5. The net decrease in blood ketone bodies caused by vasopressin was similar when somatostatin was infused simultaneously (1 nmol/kg body wt. per min). 6. Hepatic ketone bodies were significantly decreased by vasopressin, as was the 3-hydroxybutyrate/acetoacetate ratio. A small increase in the hepatic concentration of several glycolytic intermediates also occurred. 7. Vasopressin did not decrease the ketonaemia produced by infusions of octanoate or long-chain triacylglycerol in rats that had been pre-treated with the anti-lipolytic agent 3,5-dimethylpyrazole. 8. In comparison with vasopressin, the infusion of adrenaline or glucose had much smaller effects in decreasing the ketonaemia of starvation, despite the 4-fold increase in plasma insulin, at 10 min, with the glucose infusion. 9. The primary metabolic effect of vasopressin in the starved rat appears to be that of decreased supply of non-esterified fatty acid to the liver. It is suggested that vasopressin has a direct anti-lipolytic effect in adipose tissue.

Hormones of the posterior pituitary (vasopressin, oxytocin), in addition to their accepted physiological functions, exert potent effects on hepatic metabolism (Hems, 1979). A well-established effect of vasopressin is the stimulation of hepatic glycogenolysis (Hems *et al.*, 1978a; Hue *et al.*, 1981), secondary to the activation of glycogen phosphorylase *a* (Hems *et al.*, 1975; Kirk *et al.*, 1979; Hers & Van Schaftingen, 1982). Unlike glucagon, which also has the above property, vasopressin stimulates hepatic glycolysis (Williamson *et al.*, 1980; Hue *et al.*, 1981). This has been attributed to activation of pyruvate dehydrogenase (Hems *et al.*, 1978b) and phosphofructokinase, the latter as a result of the accumulation of fructose 2,6-bisphosphate, a potent activator of this enzyme (Hers & Van Schaftingen, 1982). In addition, vasopressin has several unique effects on hepatic lipid metabolism (see Sugden & Williamson, 1981). These include the inhibition of ketogenesis from oleate, stimulation of the esterification and oxidation of this fatty acid (Williamson *et al.*, 1980; Sugden *et al.*, 1980) and accelerated turnover of phosphatidylinositol (Kirk *et al.*, 1979, 1981). Most of these effects are associated with the redistribution of Ca^{2+} within the liver cell [cyclic AMP (and/or cyclic GMP)-independent]. The possible clinical implications of these findings in relation to the stressed fed state have been discussed (Williamson *et al.*, 1980; Sugden & Williamson, 1981).

In contrast with the range of effects seen in the fed (glycogen-replete) liver, the only well-established effect of vasopressin *in vitro* in the starved liver is the stimulation of gluconeogenesis (Hems & Whitton, 1973; Siess *et al.*, 1978; Whitton *et al.*, 1978). Lipid metabolism, however, appears to be unaffected by vasopressin. Ketogenesis *in vitro* from endogenous (Siess *et al.*, 1978) or exogenous fatty acid (Williamson *et al.*, 1980) is not inhibited, esterification of oleate is similarly unaffected (Williamson *et al.*, 1980), and pyruvate dehydrogenase is not activated (Denton *et al.*, 1981), nor is phosphofructokinase (Hue *et al.*, 1981). Many of the observed effects of vasopressin on lipid metabolism

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in the fed state may therefore be secondary to stimulation of glycogenolysis with accelerated glycolysis (Williamson *et al.*, 1980; Sugden & Williamson, 1981). However, vasopressin may have effects on hepatic metabolism in the whole animal which are unlikely to be seen under the conditions of restricted substrate supply used in hepatocyte studies. By analogy, adrenaline appears to have either no (Siess *et al.*, 1978) or minor (Burrin *et al.*, 1982) stimulatory effects on ketogenesis from starved rats, yet *in vivo* it decreases the ketonaemia of starvation, apparently by a hepatic mechanism (Sugden *et al.*, 1982).

The effects of vasopressin *in vivo* in the fed rat have been investigated by Hems *et al.* (1975), using a single intraperitoneal dose of the hormone. They found that a substantial increase in blood glucose concentration, with a subsequent increase in insulin concentration, were the predominant effects. A more recent study, also using fed rats, showed similar results (Triscari & Sullivan, 1981). The above approach to investigating the action of vasopressin *in vivo* has some shortcomings, including the use of single large doses of a hormone which has a short half-life (Janáky *et al.*, 1982). The aim of the present study was to examine the metabolic effects of vasopressin in the starved rat by using constant infusions of the hormone, and, in particular, to see whether its lack of effect on hepatic ketogenesis in the starved state *in vitro* also applies *in vivo*.

Materials and methods

Animals

Male Wistar rats (210–260 g) were fed *ad libitum* on Oxoid breeding diet for rats and mice (Oxoid, London S.E.1, U.K.) and were subjected to a 12h-light/12h-dark cycle, the light period starting at 07:00h. Rats were starved from 09:00h and the experiments were conducted the following day from 09:00 to 13:00h.

Experimental

Rats were anaesthetized with Nembutal (60 mg/kg body wt.; solution in 0.15 M-NaCl) and polythene cannulae were placed in the external jugular vein and the internal carotid artery (cannulae dimensions, internal $\times$ external, 0.40 mm $\times$ 0.80 mm for the vein and 0.58 mm $\times$ 0.96 mm for the artery; Portex, Hythe, Kent, U.K.). All solutions were infused via the jugular vein, and at appropriate times blood (0.4 ml) was withdrawn from the carotid artery and either deproteinized in HClO₄ or heparinized for the separation of plasma. Care was taken to maintain the plasma volume of the rats throughout the experiment. In studies where lipid was infused, an intraperitoneal injection of 3,5-dimethylpyrazole (50 mg/kg body wt.; solution in 0.15 M-NaCl) was given 40 min before the start of the infusion.

Infusion protocols

Hormones were diluted in 0.15 M-NaCl (saline) and, unless otherwise stated, infused at a rate of 33 μ l/min with a syringe pump (Sage Instruments, New York, NY, U.S.A.). Vasopressin studies were initiated with a loading dose of 150 pmol/kg body wt. (0.4 ml) and then infused at 50 pmol/kg body wt. per min (1 pmol = 1 ng = 0.4 munit). Control rats were infused in an identical manner with 0.15 M-NaCl. Somatostatin was infused at a rate of 1 nmol/kg body wt. per min throughout, and adrenaline was infused at 50 nmol/kg body wt. for 1 min and at 25 nmol/kg body wt. per min thereafter. Glucose was infused at 200 μ mol/kg body wt. for the first 4 min (0.5 M solution in 0.15 M-NaCl) and at 10% of this rate and volume thereafter. Sodium octanoate (0.15 M) was given as an initial bolus (300 μ mol/kg body wt.) and then infused at 25 μ mol/kg body wt. per min. The long-chain triacylglycerol (Intralipid 20%) was infused at a rate of 400 μ l/kg body wt. per min for 6.5 min, followed by saline infusion at the standard rate. In addition, these rats received heparin (150 units/kg body wt.) at the start of the infusion and 50 units/kg body wt. every 10 min. When given in conjunction with other treatments, vasopressin was administered as described above, being combined where necessary with the other infusion material. Arterial blood-pressure recordings were made with a pressure amplifier (Lexington Instruments, Waltham, MA, U.S.A.).

Liver and blood metabolite measurements

Blood samples were immediately deproteinized in 4 vol. of 6% (w/v) HClO₄ and the liver was freeze-clamped (Wollenberger *et al.*, 1960) immediately after the last blood sample was taken. The frozen tissue was treated by the method of Williamson *et al.* (1967a). Metabolites were measured in neutralized HClO₄ extracts by the following enzymic methods: acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962); glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963); ADP and AMP (Adam, 1963); glycerol 3-phosphate (Hohorst, 1963a); phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate (Czok & Eckert, 1963); citrate (Dagley, 1963); L-malate (Hohorst, 1963b); L-alanine (Williamson *et al.*, 1967b); L-glutamate (Bernt & Bergmeyer, 1963); L-aspartate (Pfleiderer, 1963); and 2-oxoglutarate (Bergmeyer & Bernt, 1963). Heparinized blood samples were centrifuged at 2000 g for 5 min, and the plasma was removed and then frozen. Non-esterified fatty acids were measured in these samples by the enzymic method of Shimizu *et al.* (1979), with oleate as the standard. Plasma insulin was measured by radioimmunoassay, with charcoal phase separation as described

by Albano *et al.* (1972). Statistical significance of differences was assessed with Student's unpaired *t* test. Results are given as means $\pm$ S.E.M., with the numbers of observations either given in parentheses or indicated in the Tables and Figures.

Materials

Enzymes and coenzymes were from Boehringer Corp. (London); [arginine]vasopressin (grade VIII) and somatostatin were from Sigma; adrenaline and octanoic acid were from BDH Chemicals; Intralipid was from KabiVitrum, London W.5, U.K.; sodium heparin was from Boots, Nottingham, U.K.; and 3,5-dimethylpyrazole was from Fluka, Buchs, Switzerland. Other chemicals were of reagent grade.

Results and discussion

Effect of vasopressin infusion on blood metabolite concentrations

Metabolic studies with vasopressin *in vitro* have used concentrations of the order of 10 nM. The infusion rate for the present experiments was chosen after considering the plasma volume, short half-life of vasopressin, particularly its rapid clearance by the liver (Little *et al.*, 1966; Errington & Rocha E Silva, 1972; Janáky *et al.*, 1982), and the results from other studies using similar infusion protocols where plasma vasopressin concentrations were monitored (Morton *et al.*, 1982). The plasma vasopressin concentration in the present study was estimated to be in the range 1–5 nM.

The infusion of vasopressin into starved anaesthetized rats resulted in an immediate decrease in the blood ketone-body concentration (1.07 mM in 10 min; Fig. 1). This change was accompanied by a significant increase in glucose (0.91 mM at 5 min), which returned to near pre-infusion values over 40 min. Increases in lactate and pyruvate were also observed, amounting to a 2-fold increase in 10 min. The lactate/pyruvate ratio was unchanged. Saline-infused control rats showed only a small and non-significant increase in glucose and lactate during the infusion period (Fig. 1).

The increase in blood glucose is consistent with a stimulation of hepatic gluconeogenesis (Hems & Whitton, 1973; Siess *et al.*, 1978; Whitton *et al.*, 1978), with a possible contribution from residual hepatic glycogen. Possible explanations for the decreased ketonaemia after infusion of vasopressin include: (a) inhibition of hepatic ketogenesis, despite its lack of effect in the starved state *in vitro* (Siess *et al.*, 1978; Williamson *et al.*, 1980); (b) increased peripheral utilization of ketone bodies; (c) decreased fatty acid supply to the liver, owing to either direct effects of vasopressin (vasoconstriction or inhibition of lipolysis) or secondary effects such as the release of insulin in response to the transient increase in

Fig. 1. *Effect of vasopressin infusion on blood metabolite concentrations in 24 h-starved rats*

Starved rats were infused with either vasopressin (50 pmol/kg body wt. per min after an initial bolus of 150 pmol/kg body wt.) or saline (0.15 M-NaCl), and metabolite concentrations were measured at the times indicated. The results are shown as means $\pm$ S.E.M. for six vasopressin infusions (■) and six saline infusions (□). Significant differences between means were assessed by using Student's *t* test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

blood glucose. However, plasma insulin concentrations at 40 min were not significantly different [14 ± 4 (6) and 22 ± 2 (5) munits/l] for vasopressin and saline infusions respectively.

Effects of vasopressin on hepatic metabolite concentrations

Possible effects of vasopressin on hepatic metabolism were examined by freeze-clamping the liver

10 min after starting the vasopressin infusion. There was no significant change in the hepatic concentration of glucose after the infusion of vasopressin, but there were significant increases in the concentrations of pyruvate, 3-phosphoglycerate and 2-oxoglutarate, as well as small increases in several other glycolytic intermediates (Table 1). The total liver ketone bodies were decreased by 55%. In addition, the mitochondrial redox state was more oxidized, as shown by the decreased 3-hydroxybutyrate/acetoacetate ratio (Table 1). The changes in blood metabolite concentrations in these rats showed similar results to those in Fig. 1. In these rats, however, the 3-hydroxybutyrate/acetoacetate ratio was significantly lowered by vasopressin treatment, reflecting the changes seen in the liver (Table 2).

An important finding was that, 10 min after starting the vasopressin infusion, plasma non-esterified fatty acids had decreased by nearly 60% (Table 2). Unlike the effects of vasopressin administration in fed rats (Hems *et al.*, 1975; Triscari & Sullivan, 1981), this decrease was not accompanied by any increase in plasma insulin concentrations (Table 2). Plasma insulin and non-esterified fatty acid concentrations at 40 min were also low in vasopressin-infused rats (results not shown). The effect of vasopressin, to decrease rapidly non-esterified fatty acid supply to the liver, would explain several changes seen in the concentrations of liver and blood metabolites. In the starved liver, reducing equivalents from the mitochondrial oxidation of fatty acid are moved to the cytosol to support gluconeogenesis, while a proportion of acetyl-CoA is diverted into ketogenesis. In the face of decreased supply of non-esterified fatty acid to the liver, and the increased energy demands of stimulated gluconeogenesis, more acetyl-CoA would be oxidized in the tricarboxylic acid cycle to provide the necessary reducing equivalents and ATP, with less carbon diverted to ketogenesis. The decreased supply of oxidizable substrate to the liver in the presence of vasopressin would also explain the more oxidized hepatic redox couples. In the cytosol, this would tend to decrease flux through glyceraldehyde 3-phosphate dehydrogenase in the direction of gluconeogenesis and would explain the accumulation of glycolytic intermediates below this point, and the decline in blood glucose concentrations after the initial increase (see Fig. 1).

Effect of vasopressin on the metabolism of infused lipid

A model that has been used to study the aspects of hepatic ketogenesis *in vivo*, independent of endogenous substrate supply, incorporates the administration of an anti-lipolytic agent (Gerritsen & Dulin, 1965), followed by the infusion of either

short- (Williamson *et al.*, 1974), medium- (McGarry & Foster, 1971) or long-chain fatty acids (as triacylglycerol; Axelrod *et al.*, 1979a). With this approach, rats were first treated with 3,5-dimethylpyrazole and then infused with either octanoate or long-chain triacylglycerol (Intralipid), the latter in the presence of heparin to stimulate lipoprotein lipase.

In rats treated with 3,5-dimethylpyrazole alone, blood ketone bodies decreased within 40 min to 0.4 mM and only increased slightly to 0.6 mM over the infusion period. Infusion of octanoate together with 3,5-dimethylpyrazole resulted in blood ketone-body concentrations similar to those seen in starvation (Fig. 2a). Vasopressin, however, caused no inhibition of the ketonaemia generated by this treatment. In fact, a small but significant increase in

Table 1. *Effect of vasopressin infusion on the hepatic metabolite content of 24 h-starved rats*

Rats were infused with vasopressin (50 pmol/kg body wt. per min after an initial bolus of 150 pmol/kg body wt.) and the livers freeze-clamped at 10 min. Metabolite concentrations were measured as described in the Materials and methods section. Results are shown as means $\pm$ S.E.M. for *n* rats. Significance of differences between means was assessed with Student's *t* test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Metabolite (mM)	Saline infusion <i>n</i> = 7	Vasopressin infusion <i>n</i> = 8
Glucose	4.41 $\pm$ 0.30	4.78 $\pm$ 0.33
Glucose 6-phosphate	0.088 $\pm$ 0.023	0.060 $\pm$ 0.009
Glycerol 3-phosphate	0.192 $\pm$ 0.032	0.160 $\pm$ 0.021
3-Phosphoglycerate	0.299 $\pm$ 0.023	0.522 $\pm$ 0.056**
2-Phosphoglycerate	0.138 $\pm$ 0.013	0.194 $\pm$ 0.033
Phosphoenolpyruvate	0.285 $\pm$ 0.046	0.385 $\pm$ 0.024
Pyruvate	0.066 $\pm$ 0.007	0.097 $\pm$ 0.010*
Lactate	0.56 $\pm$ 0.10	0.75 $\pm$ 0.12
2-Oxoglutarate	0.040 $\pm$ 0.008	0.065 $\pm$ 0.006*
Malate	0.153 $\pm$ 0.020	0.144 $\pm$ 0.014
Aspartate	0.88 $\pm$ 0.09	1.09 $\pm$ 0.09
Glutamate	1.36 $\pm$ 0.05	0.76 $\pm$ 0.11***
Alanine	0.217 $\pm$ 0.013	0.357 $\pm$ 0.071
Citrate	0.317 $\pm$ 0.050	0.316 $\pm$ 0.025
3-Hydroxybutyrate	1.63 $\pm$ 0.08	0.73 $\pm$ 0.10***
Acetoacetate	0.89 $\pm$ 0.10	0.79 $\pm$ 0.10
Total ketone bodies	2.53 $\pm$ 0.11	1.53 $\pm$ 0.11***
Lactate/pyruvate	9.07 $\pm$ 2.22	7.99 $\pm$ 1.39
3-Hydroxybutyrate/ acetoacetate	2.01 $\pm$ 0.29	1.06 $\pm$ 0.20*
Malate/aspartate	0.18 $\pm$ 0.03	0.14 $\pm$ 0.01
ATP	1.37 $\pm$ 0.10	1.63 $\pm$ 0.11
ADP	1.79 $\pm$ 0.06	1.83 $\pm$ 0.06
AMP	0.94 $\pm$ 0.05	0.88 $\pm$ 0.05
Total adenine nucleotides	4.11 $\pm$ 0.08	4.30 $\pm$ 0.07

ketone bodies was observed in the rats receiving octanoate and vasopressin. The blood glucose concentration increased slightly, but there was little change in the concentrations of lactate or pyruvate (results not shown). The conversion of octanoate into ketone bodies by-passes several possible regulatory steps. It is not re-esterified in liver (McGarry & Foster, 1971), neither does it require the carnitine acyltransferase system for transport into the mitochondrial matrix (see Williamson, 1979). The effects of vasopressin were therefore examined when ketonaemia was supported by long-chain fatty acids provided by infusing long-chain triacylglycerol.

To achieve similar ketone-body concentrations, within 10 min, to those seen in starvation or with octanoate infusion, it was necessary to use infusion rates of long-chain triacylglycerol (see the Materials and methods section) which precluded reaching a steady state in short-term infusions. Under these conditions, vasopressin actually caused a significant increase in ketonaemia during the first 10 min (Fig. 2b).

These results with infusion of octanoate and long-chain triacylglycerol suggest that in the presence of ample precursor supply to the liver vasopressin is unable to exert its anti-ketogenic effect, and therefore its site of action is likely to be extrahepatic. However, the possibility that the anti-lipolytic agent or the infused fatty acid have effects in liver that mask or abolish those of vasopressin cannot be excluded.

Effects of simultaneous infusion of somatostatin and vasopressin

To verify that the effects seen with vasopressin

were not secondary to stimulation of insulin release, rats were first pre-infused with somatostatin to inhibit insulin secretion, as described by Nosadini *et al.* (1980), and then simultaneously infused with vasopressin and somatostatin. The results of the pre-infusion and the first 10 min of vasopressin infusion are shown in Table 3. Ketone-body concentrations decreased by 0.75 ± 0.13 (5) mM in 10 min, compared with 1.07 mM (Fig. 1) and 0.96 mM (Table 1) in the absence of somatostatin. However, the ketone-body concentration in rats receiving somatostatin alone increased by 0.32 ± 0.08 (5) mM between 10 and 20 min. Therefore there was no net difference in the effect of vasopressin on blood ketone-body concentrations in the presence of somatostatin. As expected, plasma insulin concentrations at 10 and 20 min (first 10 min of vasopressin infusion) were very low (range 0–7 munits/l) for rats infused with either somatostatin or somatostatin plus vasopressin. During this same period, the concentration of plasma non-esterified fatty acids increased slightly in the somatostatin-infused rats, and decreased by 48% in those receiving both somatostatin and vasopressin (Table 3).

Effect of adrenaline and glucose infusions on blood metabolite concentrations

It has been shown that the subcutaneous injection of adrenaline decreases the ketonaemia of starvation (Sugden *et al.*, 1982). This effect appears related to alterations in hepatic gluconeogenesis and was shown to be independent of fatty acid supply to the liver. Adrenaline provides a useful comparison in

Table 2. *Effect of vasopressin infusion on blood metabolite concentrations in 24 h-starved rats*

The rats were those used in the freeze-clamping experiment (see Table 1 and text for details). Results are given as means $\pm$ S.E.M. for seven saline and eight vasopressin infusions. The change at 10 min was calculated from data for individual rats. ** $P < 0.01$; *** $P < 0.001$.

Metabolite (mM)		Time (min)		Mean change in 10 min
		0	10	
Glucose	Saline	3.87 ± 0.20	3.82 ± 0.22	-0.05 ± 0.11
	Vasopressin	3.73 ± 0.26	4.50 ± 0.34	$+0.76 \pm 0.21^{**}$
Lactate	Saline	0.37 ± 0.04	0.47 ± 0.08	$+0.10 \pm 0.05$
	Vasopressin	0.43 ± 0.07	0.78 ± 0.11	$+0.35 \pm 0.05^{**}$
Pyruvate	Saline	0.050 ± 0.007	0.039 ± 0.005	-0.011 ± 0.005
	Vasopressin	0.049 ± 0.007	0.070 ± 0.008	$+0.024 \pm 0.006^{***}$
Ketone bodies	Saline	2.24 ± 0.43	2.38 ± 0.16	$+0.15 \pm 0.06$
	Vasopressin	2.49 ± 0.86	1.52 ± 0.23	$-0.96 \pm 0.10^{***}$
Lactate/pyruvate	Saline	8.57 ± 1.94	12.7 ± 2.6	$+4.17 \pm 1.43$
	Vasopressin	9.65 ± 2.02	10.8 ± 2.1	$+1.40 \pm 1.50$
3-Hydroxybutyrate/acetoacetate	Saline	1.75 ± 0.14	1.98 ± 0.30	$+0.23 \pm 0.09$
	Vasopressin	1.93 ± 0.13	1.21 ± 0.10	$-0.71 \pm 0.19^{***}$
Non-esterified fatty acids	Saline	0.96 ± 0.08	0.94 ± 0.08	-0.02 ± 0.04
	Vasopressin	1.02 ± 0.06	0.41 ± 0.03	$-0.60 \pm 0.06^{***}$
Insulin (munits/l)	Saline	7.2 ± 1.4	4.4 ± 1.3	-2.8 ± 1.6
	Vasopressin	13.7 ± 3.2	6.6 ± 1.9	-7.1 ± 2.6

the present study, as several of its effects are similar to that of vasopressin, including the stimulation of hepatic glycogenolysis, and, in the starved state, stimulation of gluconeogenesis (see Sugden & Williamson, 1981). In addition, both vasopressin and adrenaline have powerful pressor effects (see below).

Blood glucose and lactate increased more markedly in response to adrenaline than with vasopressin (Fig. 3). Pyruvate increased in parallel to lactate (results not shown). Although the ketone-body concentration decreased after adrenaline administration, the initial rate of change was only 23% of that seen with vasopressin (0.25 mM/10 min, cf. 1.07 mM/10 min). However, this rate of decrease with adrenaline would, if sustained, be sufficient to account for the decrease of ketonaemia (0.6 mM) reported by Sugden *et al.* (1982) to occur after 60 min. Adrenaline tended to decrease the plasma non-esterified fatty acid concentration (results not shown), though the change was not significant, which is unexpected in light of the reported lipolytic action of catecholamines (see Alberti *et al.*, 1978). It is possible that acute infusion of adrenaline may actually inhibit lipolysis by a mechanism involving the α -receptor. However, although α -agonists inhibit lipolysis in adipocytes from various species, the effect in rat adipocytes is not established (Schimmel, 1979).

Despite the similarity in pressor response and the greater increase in blood glucose, lactate and pyruvate seen with adrenaline, vasopressin caused a much larger and more immediate decrease in ketone-body concentration, which suggests that the mechanism for vasopressin action in depressing ketonaemia is not directly related to these changes alone. Another difference between the effects of the two hormones is that the ketonaemia sustained by exogenously supplied fatty acid, either medium- or long-chain, was not reversed by vasopressin, yet it was by adrenaline (Sugden *et al.*, 1982). It is therefore reasonable to conclude that the two hormones lower blood ketone-body concentrations by different mechanisms.

Administration of vasopressin was accompanied by an increase in blood glucose (Fig. 1). To test whether such an increase might be sufficient to elicit the response seen with vasopressin, glucose was infused at a rate of 200 μ mol/kg body wt. per min into starved rats for 4 min and at 20 μ mol/kg body wt. per min thereafter. The rise in blood glucose (2.2 mM) after the glucose infusion caused the ketone-body concentration to decrease linearly from 5 min, at an initial rate one-half of that seen with vasopressin (Fig. 3). The plasma non-esterified fatty acid concentration decreased from 0.88 ± 0.06 (5) to 0.65 ± 0.05 (5) mM in the first 10 min, and insulin concentrations increased 4-fold [11 ± 2 (5) to

Fig. 2. Effect of vasopressin on the blood ketone-body concentration in 24 h-starved rats after infusion of medium- or long-chain lipid

Starved rats were pre-treated with 3,5-dimethylpyrazole and infused with either octanoate (a) or long-chain triacylglycerol (b) as described in the Materials and methods section. The vasopressin infusion (50 pmol/kg body wt. per min, after an initial bolus of 150 pmol/kg body wt.) was started at 10 min. The results are shown as means $\pm$ S.E.M. for four infusions with vasopressin (■) and four saline controls (□). Significant differences between means were assessed with Student's *t* test. * $P < 0.05$.

45 ± 9 (5) munits; $P < 0.01$] during this time. These findings are consistent with inhibition of lipolysis after insulin release, with subsequent decreased substrate supply to the liver and hence diminished ketogenesis. The important finding is that, despite the 4-fold increase in the plasma insulin concentration, glucose infusion caused a smaller decrease in the blood ketone-body and non-esterified fatty acid concentrations than did vasopressin, lending further support to the view that the latter may act directly to decrease lipolysis in adipose tissue.

Table 3. *Effect of vasopressin on blood metabolite concentrations in rats infused with somatostatin*

Rats were infused with somatostatin (1 nmol/kg body wt. per min) for 10 min and were then infused with both vasopressin (150 pmol/kg body wt. bolus, then 50 pmol/kg body wt. per min) and somatostatin for a further 30 min. The results of the preinfusion and the first 10 min of the simultaneous infusion are shown and are expressed as means $\pm$ S.E.M. for five rats for each treatment. The significance of differences refers to the mean change (10–20 min) in metabolite concentration between treatments for individual rats. Significant differences were assessed by using Student's *t* test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Metabolite (mm)	Infusion	Time (min)			Mean change (10–20 min)
		0	10	20	
Glucose	Somatostatin	4.10 $\pm$ 0.12	4.22 $\pm$ 0.13	3.97 $\pm$ 0.22	–0.15 $\pm$ 0.11
	Somatostatin + vasopressin	3.59 $\pm$ 0.16	3.44 $\pm$ 0.16	4.00 $\pm$ 0.12	+0.56 $\pm$ 0.10**
Lactate	Somatostatin	0.26 $\pm$ 0.04	0.28 $\pm$ 0.07	0.44 $\pm$ 0.08	+0.15 $\pm$ 0.04
	Somatostatin + vasopressin	0.47 $\pm$ 0.08	0.48 $\pm$ 0.06	0.59 $\pm$ 0.13	+0.11 $\pm$ 0.10
Pyruvate	Somatostatin	0.050 $\pm$ 0.008	0.045 $\pm$ 0.009	0.047 $\pm$ 0.011	+0.002 $\pm$ 0.011
	Somatostatin + vasopressin	0.060 $\pm$ 0.011	0.041 $\pm$ 0.004	0.054 $\pm$ 0.004	+0.012 $\pm$ 0.006
Ketone bodies	Somatostatin	2.22 $\pm$ 0.63	2.39 $\pm$ 0.39	2.71 $\pm$ 0.65	+0.32 $\pm$ 0.08
	Somatostatin + vasopressin	2.74 $\pm$ 0.33	2.78 $\pm$ 0.24	2.03 $\pm$ 0.33	–0.75 $\pm$ 0.13***
Lactate/pyruvate	Somatostatin	5.6 $\pm$ 1.2	10.1 $\pm$ 3.5	11.5 $\pm$ 2.9	+1.4 $\pm$ 2.6
	Somatostatin + vasopressin	9.6 $\pm$ 1.6	11.6 $\pm$ 1.1	11.4 $\pm$ 2.4	–0.2 $\pm$ 2.9
3-Hydroxybutyrate/ acetoacetate	Somatostatin	1.59 $\pm$ 0.38	1.66 $\pm$ 0.39	1.91 $\pm$ 0.65	+0.25 $\pm$ 0.13
	Somatostatin + vasopressin	1.86 $\pm$ 0.19	1.99 $\pm$ 0.25	1.17 $\pm$ 0.15	–0.82 $\pm$ 0.35*
Non-esterified fatty acids	Somatostatin	—	0.82 $\pm$ 0.06	0.86 $\pm$ 0.05	+0.04 $\pm$ 0.03
	Somatostatin + vasopressin	—	0.83 $\pm$ 0.06	0.43 $\pm$ 0.06	–0.40 $\pm$ 0.06***
Insulin (munits/l)	Somatostatin	—	3.1 $\pm$ 1.2	3.0 $\pm$ 1.0	—
	Somatostatin + vasopressin	—	1.1 $\pm$ 0.7	0.2 $\pm$ 0.2	—

Pressor activity

The rate of infusion of vasopressin required to cause the rapid decrease in blood ketone-body concentration was in the range where maximum pressor effects have been reported (Morton *et al.*, 1982). This was confirmed here, with initial blood pressures rising from 112 $\pm$ 3 (10) to 150 $\pm$ 1 (10) mmHg (*P* < 0.001) within the first 2 min of infusion. Adrenaline caused a similar increase [119 $\pm$ 5 (6) to 160 $\pm$ 4 (6) mmHg, *P* < 0.001] in the same period. Although vasopressin may have selective pressor effects in certain tissues (Saameli, 1968), it is unlikely that its effects are due to a general systemic vasoconstriction. Hems *et al.* (1976) found that vasopressin concentrations greater than 10 nM were required to cause vasoconstriction in the perfused rat liver. If this were a factor in the effects seen here, some indication of anoxia (increased lactate/pyruvate ratio in liver) might be expected. This was not seen (Table 2). It is possible that any vasoconstriction in the liver, or indeed in other tissues, is counteracted by the increased cardiac output in response to vasopressin.

Clinical implications

Several hormones, including those from the posterior pituitary and the catecholamines, have been implicated in the catabolic metabolism seen in a variety of disease states (Hems, 1979). The release

of vasopressin, either appropriately or inappropriately, occurs in a range of clinical situations, including haemorrhage, diabetes, head trauma, and after the use of anti-neoplastic drugs and thiazide diuretics (see Moses *et al.*, 1976). The present finding in regard to the apparent anti-lipolytic effect of vasopressin has implications in relation to the metabolic changes that occur in these states, and in particular may link the following observations in regard to sepsis.

(a) Infection in starved rats produces a sharp decline in the blood ketone-body and non-esterified fatty acid concentrations (Neufeld *et al.*, 1976; Beisel & Wannemacher, 1980). (b) This effect is not seen in hypophysectomized rats, though it was still apparent in adrenalectomized and thyroidectomized animals (Neufeld *et al.*, 1980). (c) Sepsis and septic shock in monkeys and dogs gives rise to large increases in the plasma vasopressin concentrations (Wilson *et al.*, 1981). Although insulin and other hormones may play a role in the above effects, it is reasonable to suggest that vasopressin may be responsible, at least in part, for the metabolic disturbances seen during sepsis. The present findings may help to explain why there is often a failure to achieve raised blood ketone-body concentrations in various other disease and injury states associated with low energy intake (Smith *et al.*, 1975; Conyers *et al.*, 1979; Rich & Wright, 1979).

Fig. 3. Effect of adrenaline, glucose and vasopressin infusions on blood metabolite concentrations in 24-h starved rats

Rats were infused with adrenaline, glucose or vasopressin. For details see the Materials and methods section. The vasopressin results from Fig. 1 have been included for comparison. The results are shown as means + S.E.M. for six adrenaline (O), five glucose (●) and six vasopressin infusions (■).

Conclusions

The primary metabolic effect of vasopressin in the starved rat was a rapid decrease in ketonaemia, with an associated decrease in the plasma concentration of non-esterified fatty acids. As no increase in plasma insulin accompanied these changes, it is suggested that vasopressin may affect fatty acid release from adipose tissue in a manner similar to insulin and prostaglandin E_2 (Hales *et al.*, 1978; Axelrod *et al.*, 1979b). Oxytocin, which has close structural similarities to vasopressin, does have mild anti-lipolytic effects *in vitro* (Rodbell, 1964). Furthermore, oxytocin has binding sites on the surface

of rat adipocytes which appear to have an even greater affinity for vasopressin, as ^{125}I -labelled oxytocin is more readily displaced from its binding sites by vasopressin than by oxytocin itself (Thompson *et al.*, 1972). It is therefore postulated that vasopressin has a direct anti-lipolytic effect on the adipocyte.

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