Impairment of effects of vasopressin on [1-¹⁴C]oleate metabolism in hepatocytes from obese (*ob/ob*) mice

Melfyn W. EDWARDS,* Michael A. CAWTHORNE† and Dermot H. WILLIAMSON*

*Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K., and †Beecham Pharmaceuticals, Biosciences Research Centre, Great Burgh, Epsom, Surrey KT18 5XQ, U.K.

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In hepatocytes from lean mice vasopressin decreased ketogenesis and increased ¹⁴CO₂ production from [1-¹⁴C]oleate and glucose release; these effects were Ca²⁺-dependent. None of these effects of vasopressin were obtained with hepatocytes from obese (*ob/ob*) mice. Similarly, adrenaline did not increase ¹⁴CO₂ production in these hepatocytes, but it stimulated glucose release. Possible reasons for the impairment of vasopressin action are discussed.

Vasopressin and adrenaline exert a number of effects on rat and mouse liver, including stimulation of glycogenolysis (Ma & Hems, 1975; Hutson *et al.*, 1976; Hems *et al.*, 1978; Ma *et al.*, 1979), activation of phosphorylase (Hems *et al.*, 1975), enhancement of gluconeogenesis (Hutson *et al.*, 1976; Whitton *et al.*, 1978) and, in mouse, inhibition of lipogenesis (Ma & Hems, 1975). Livers from genetically obese mice (*ob/ob* strain), however, show resistance to the effects of vasopressin and adrenaline on lipogenesis (Hems & Ma, 1976; Ma *et al.*, 1979).

Vasopressin has been shown to increase oleate esterification and to inhibit ketogenesis from oleate in hepatocytes from fed rats (Williamson *et al.*, 1980). This inhibition is dependent on the presence of Ca²⁺ in the medium and appears to be mainly due to increased oxidation of oleate to CO₂ (Sugden *et al.*, 1980a). Adrenaline is also able to increase the conversion of [1-¹⁴C]oleate into ¹⁴CO₂, but this is not accompanied by a decrease in ketogenesis (Sugden *et al.*, 1980b). Both hormones increase the release of glucose to the medium.

The work reported here addresses itself to the following questions. Does vasopressin have similar effects on oleate metabolism in hepatocytes from normal mice to those reported for rat hepatocytes and do hepatocytes from obese mice exhibit resistance to vasopressin in respect of oleate metabolism?

The novel finding in the present work is that the livers of obese mice appear to be resistant to all the established effects of vasopressin.

Experimental

Female CFLP mice (Hacking and Churchill, Huntingdon, Cambs., U.K.) or male C57 B1 6J mice

(OLAC, Bicester, U.K.) aged 12–18 weeks were fed on Oxoid breeders diet (H. C. Styles, Bewdley, Worcs., U.K.). Obese mice were homozygous for the *ob* gene. Lean mice were of mixed genotype, i.e. heterozygous or homozygous for the normal allele. For the experiments involving diet restriction, *ob/ob* mice were selected at 5 weeks of age and were given 2.5 g of diet per day (1 g at 08:30 h and 1.5 g at 16:00 h). On this regime they attained a stable weight of 27–32 g, which was identical with lean littermates fed *ad libitum*. However, although the body weight of the mice was the same, the mice bearing the *ob/ob* genotype are likely to have greater fat deposits than the lean littermates (Alonso & Maren, 1955; Cox & Powley, 1977).

Isolated hepatocytes were prepared by perfusing the liver (Salmon *et al.*, 1974) with collagenase (25 mg/80 ml of medium; Berry & Friend, 1969; Krebs *et al.*, 1974). Hepatocytes were prepared in the absence of added Ca²⁺, but cells were washed with Krebs–Henseleit (1932) saline containing Ca²⁺. Preparation of hepatocytes was commenced between 09.30 h and 10.30 h.

The procedure for the incubation of hepatocytes and measurement of esterification of [1-¹⁴C]oleate and its conversion into CO₂ were as described by Whitelaw & Williamson (1977). Where indicated Ca²⁺ was omitted from the incubation medium, but a Ca²⁺-chelator was not added. The substrates for lipogenesis were 15 mM-glucose and 10 mM-lactate. Lipogenesis was measured with ³H₂O by the method described by Harris (1975). The following metabolites were determined in the neutralized HClO₄ extracts by enzymic methods: glucose (Slein, 1963); acetoacetate and 3-hydroxybutyrate (Williamson *et al.*, 1962).

The metabolite changes between 10 and 40 min were calculated from plots of the values at 10, 20 and 40 min, which were linear, and the results expressed as $\mu\text{mol/min per g}$ fresh wt. of cells $\pm$ S.E.M., with the numbers of observations in parentheses. The fresh weight of cells was calculated by determining the dry weight and multiplying this by a factor obtained from fresh/dry-weight ratio measurements made on hepatocytes of lean and obese mice as described by Krebs *et al.* (1974).

All enzymes and coenzymes were obtained from Boehringer. [Arginine]vasopressin (grade VI) was obtained from Sigma. Adrenaline was from BDH Chemicals. Phentolamine mersylate B.P. (Rogitine) was from C.I.B.A. Laboratories, Horsham, Sussex, 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.

Results and discussion

Effects of vasopressin in hepatocytes from lean mice

The addition of vasopressin (25 nM) to hepatocytes from fed lean mice increased oxidation of [$1\text{-}^{14}\text{C}$]oleate to $^{14}\text{CO}_2$ by 40–70% (depending on the strain used), decreased the rate of ketogenesis (21–22%) and stimulated glucose release (32–77%) (Table 1). These results are qualitatively the same as those obtained with rat hepatocytes under similar conditions (Williamson *et al.*, 1980; Sugden *et al.*, 1980a).

In contrast with the findings with rat hepatocytes (Williamson *et al.*, 1980; Sugden *et al.*, 1980a) vasopressin did not increase esterification in the hepatocytes from lean mice. Another apparent species difference occurs with regard to the effects of vasopressin on lipogenesis. The hormone inhibits lipogenesis in livers of intact mice and in perfused mouse livers (Ma & Hems, 1975; Hems & Ma, 1976). We have demonstrated this inhibition with vasopressin in mouse hepatocytes (Table 1), whereas the hormone has no significant inhibitory effect on hepatic lipogenesis in rat (Kirk & Hems, 1979).

Impairment of the effects of vasopressin in hepatocytes from *ob/ob* mice

When vasopressin was added to hepatocytes from *ob/ob* mice there was no alteration in the rates of any parameter measured (lipogenesis, ketogenesis, glucose release, oxidation of [$1\text{-}^{14}\text{C}$]oleate to $^{14}\text{CO}_2$ or its esterification). Resistance to the inhibitory effect of vasopressin on lipogenesis has been shown previously in *ob/ob* mice, *in vivo* and in perfused livers (Hems & Ma, 1976).

Diet restriction of *ob/ob* mice so that they did not increase their body weight above that of lean littermates did not restore the vasopressin effect on

Table 1. Effect of vasopressin on [$1\text{-}^{14}\text{C}$]oleate esterification, $^{14}\text{CO}_2$ production, ketogenesis, glucose release and $^3\text{H}_2\text{O}$ incorporation into fatty acid in mouse hepatocytes. For details see the Experimental section. [$1\text{-}^{14}\text{C}$]Oleate (1 mM) was present in all experiments, and the final concentration of vasopressin where added was 25 nM. The results are means $\pm$ S.E.M. with the numbers of observations in parentheses. Values that are significantly different from those for oleate alone are indicated: * $P < 0.05$; ** $P < 0.01$.

Strain of mice	Additions	Ketogenesis ($\mu\text{mol/g}$ of liver per min)	Glucose release ($\mu\text{mol/g}$ of liver per min)	$^{14}\text{CO}_2$ production (nmol of oleate oxidized/g of liver per min)	Oleate esterification (nmol/g of liver per min)	$^3\text{H}_2\text{O}$ incorporation (μmol of $^3\text{H}_2\text{O/g}$ of liver per h)
CFLP +/+	None	0.45 $\pm$ 0.04 (11)	0.89 $\pm$ 0.05 (8)	24 $\pm$ 3 (8)	142 $\pm$ 12 (6)	12.5 $\pm$ 2.6 (9)
	Vasopressin	0.35 $\pm$ 0.04 (11)**	1.53 $\pm$ 0.10 (8)**	41 $\pm$ 3 (8)**	144 $\pm$ 10 (6)	8.0 $\pm$ 2.0 (9)**
C57B1 +/?	None	0.83 $\pm$ 0.11 (6)	1.48 $\pm$ 0.18 (6)	29 $\pm$ 2.6 (6)	—	—
	Vasopressin	0.66 $\pm$ 0.07 (6)*	1.96 $\pm$ 0.30 (6)*	41 $\pm$ 3.6 (6)**	—	—
C57B1 <i>ob/ob</i>	None	0.32 $\pm$ 0.04 (6)	0.50 $\pm$ 0.16 (6)	14 $\pm$ 1.9 (6)	92 $\pm$ 11 (6)	12.7 $\pm$ 4.5 (4)
	Vasopressin	0.33 $\pm$ 0.04 (6)	0.53 $\pm$ 0.20 (6)	16 $\pm$ 2.9 (6)	95 $\pm$ 13 (6)	13.0 $\pm$ 4.2 (4)
C57B1 <i>ob/ob</i> , diet restricted	None	0.21 $\pm$ 0.04 (4)	1.25 $\pm$ 0.12 (4)	42 $\pm$ 7.4 (4)	—	—
	Vasopressin	0.19 $\pm$ 0.03 (4)	1.27 $\pm$ 0.14 (4)	44 $\pm$ 5.3 (4)	—	—

Table 2. Comparison of the effects of adrenaline and vasopressin on ketogenesis, $^{14}\text{CO}_2$ production and glucose release in hepatocytes from fed C57B1 mice in the absence or presence of CaCl_2

For details see the Experimental section. $[1\text{-}^{14}\text{C}]\text{Oleate}$ (1 mM) was present in all experiments and the final concentration of the hormones is given in the Table. The results are means $\pm$ S.E.M. with the numbers of observations in parentheses. Values that are significantly different from those for oleate alone are indicated: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. '– CaCl_2 ' refers to incubations in which Ca^{2+} was omitted but no Ca^{2+} -chelator was added.

Genotype	Additions	+ CaCl_2			– CaCl_2		
		Ketogenesis ($\mu\text{mol/g}$ of liver per min)	Glucose release ($\mu\text{mol/g}$ of liver per min)	$^{14}\text{CO}_2$ production (nmol of oleate oxidized/g of liver per min)	Ketogenesis ($\mu\text{mol/g}$ of liver per min)	Glucose release ($\mu\text{mol/g}$ of liver per min)	$^{14}\text{CO}_2$ production (nmol of oleate oxidized/g of liver per min)
+/?	None	0.83 ± 0.11 (6)	1.48 ± 0.18 (6)	29 ± 2.6 (6)	0.44 ± 0.01 (3)	0.73 ± 0.05 (3)	20 ± 1 (3)
	Vasopressin (25 nM)	0.66 ± 0.07 (6)*	1.96 ± 0.3 (6)*	41 ± 3.6 (6)**	0.38 ± 0.04 (3)	0.79 ± 0.09 (3)	20 ± 1 (3)
	Adrenaline (5 μM)	0.72 ± 0.13 (6)	2.20 ± 0.33 (6)*	38 ± 3.1 (6)**	0.40 ± 0.04 (3)	1.23 ± 0.1 (3)**	19 ± 2 (3)
	None	0.19 ± 0.02 (4)	0.98 ± 0.17 (4)	13 ± 2 (4)			
ob/ob	Vasopressin (25 nM)	0.20 ± 0.03 (4)	0.94 ± 0.19 (4)	15 ± 2 (4)			
	Vasopressin (250 nM)	0.17 ± 0.04 (4)	0.94 ± 0.19 (4)	15 ± 2 (4)			
	Adrenaline (5 μM)	0.22 ± 0.03 (4)	1.26 ± 0.22 (4)*	16 ± 2 (4)			
	Adrenaline (50 μM)	0.20 ± 0.04 (4)	1.21 ± 0.20 (4)*	15 ± 3 (4)			

ketogenesis, glucose release or complete oxidation of oleate (Table 1). This indicates that the impairment of the vasopressin effect is likely to be due to the *ob/ob* genotype. The basal rates of glucose release and $^{14}\text{CO}_2$ production were higher in the hepatocytes from diet restricted mice compared with those from obese mice (Table 1). Hems & Ma (1976) found that diet restriction restored the glycolytic effect of vasopressin but not its inhibitory effect on lipogenesis. Their *ob/ob* mice were obtained from an inbred colony at Imperial College, University of London, London S.W.7, U.K.

Ca²⁺ dependence of the effects of adrenaline and vasopressin in hepatocytes from lean mice

In the presence of Ca^{2+} , adrenaline had similar effects to vasopressin with respect to glucose release and $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{oleate}$ in hepatocytes from lean mice (Table 2).

The decrease in ketogenesis with adrenaline was not significant and this is also true for rat hepatocytes (Sugden *et al.*, 1980b).

In the absence of added Ca^{2+} , vasopressin had no effect on any of the parameters measured. Adrenaline stimulation of CO_2 production also appears to be Ca^{2+} -dependent, as it is in rat hepatocytes (Sugden *et al.*, 1980b). Adrenaline is able to stimulate glucose release in the absence of Ca^{2+} ; this is presumably a Ca^{2+} -independent effect mediated via cyclic AMP.

The increase in CO_2 production in the presence of adrenaline is due to α -adrenergic stimulation; no effect is seen when adrenaline is present with the α -antagonist, phentolamine [$1.80 \mu\text{M}$; 27 ± 2.2 (6) versus 29 ± 2.6 (6) for controls]. The vasopressin-stimulated increase in CO_2 production is Ca^{2+} -dependent but is not mediated via the α -receptor since the increase occurred in the presence of phentolamine [38 ± 4.7 (3) versus 26 ± 4 (3) for controls; $P < 0.01$]. Phentolamine alone had no effect on $^{14}\text{CO}_2$ production [28 ± 2.1 (3)].

Effects of vasopressin and adrenaline on ob/ob mouse hepatocytes

Hepatocytes from *ob/ob* mice were resistant to vasopressin even at the higher dose of 250 nM (Table 2). Two experiments in which the concentration was increased to $2.5 \mu\text{M}$ also showed no effects of the hormone (results not shown). Adrenaline had no effect on ketogenesis or $^{14}\text{CO}_2$ production but did increase glucose release (Table 2). It is assumed that the latter effect is mediated via the β -receptor and cyclic AMP.

Conclusions

The results reported in the present paper show that as in rat hepatocytes (Williamson *et al.*, 1980;

Sugden *et al.*, 1980a), vasopressin increased glucose release and $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ oleate and decreased ketogenesis in mouse hepatocytes. Species differences exist with respect to the effects of vasopressin on esterification and lipogenesis.

The previous observations that livers from *ob/ob* mice are resistant to the inhibitory effects of vasopressin on lipogenesis (Hems & Ma, 1976) have been confirmed in hepatocytes and extended to the other effects of the hormone on lipid catabolism. Hepatocytes from obese mice are also resistant to the Ca^{2+} -dependent effects of adrenaline. The apparent impairment of the action of vasopressin and adrenaline on hepatocytes of obese mice may be due to: (a) decreased numbers of receptors for vasopressin and α -adrenergic agonists, (b) defective couplings between receptor occupancy and mobilization of Ca^{2+} or (c) decreased availability of mobilizable Ca^{2+} .

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