STIMULATION OF [1-^{14}C]OLEATE OXIDATION TO $^{14}CO_2$ IN ISOLATED RAT HEPATOCYTES BY VASOPRESSIN: EFFECTS OF Ca^{2+}

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1. Introduction

Vasopressin and glucagon exert a number of rapid effects on the liver, which include stimulation of glycogenolysis [1,2], activation of phosphorylase [3,4] and enhancement of gluconeogenesis [5,6]. It seems likely that these hormones exert their effects in different ways, since unlike glucagon, vasopressin does not increase the hepatic cyclic AMP concentration [7] and increases glycolytic flux [8]. Glucagon (or cyclic AMP) stimulates ketogenesis [9]. This has been suggested to result from a decrease in the concentration of malonyl-CoA which inhibits carnitine acyltransferase I (EC 2.3.1.21), an enzyme required for the entry of long-chain acyl-CoA into the mitochondrion for oxidation [10,11]. The decrease in malonyl-CoA is associated with inhibition of lipogenesis [10]. Vasopressin was demonstrated to inhibit ketogenesis in hepatocytes from fed rats when oleate is the added substrate [8]. However, vasopressin does not stimulate lipogenesis [8,12]. This suggests that the antiketogenic action of vasopressin does not result from an increase in the malonyl-CoA concentration. The antiketogenic effect might in part result from the increased conversion of oleoyl-CoA into esterified products [8] and consequently less oxidation of oleoyl-CoA to acetyl-CoA. A possible effect of vasopressin on the complete oxidation of oleate was not investigated in [8]. This communication indicates that there is an intramitochondrial site of action of vasopressin, namely increased oxidation of [1-^{14}C]oleate to CO_2. This action of vasopressin is dependent on Ca^{2+} and may contribute to the antiketogenic action of the hormone.

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2. Materials and methods

2.1. *Reagents*

All enzymes and coenzymes were obtained from Boehringer Corp., London W5. [Arginine]-vasopressin (grade VIII) and glucagon were obtained from Sigma (London) Chemical Co., Poole. [1-^{14}C]Oleate and ^3H_2O were purchased from The Radiochemical Centre, Amersham.

2.2. *Preparation of hepatocytes*

Female Wistar rats (180–250 g) were fed ad libitum on standard laboratory diet. They were anaesthetized with Nembutal (60 mg/kg body wt; solution in 0.9% NaCl). Isolated hepatocytes were prepared essentially as in [13] and modified as in [14]. Preparation of hepatocytes was commenced between 9:30 and 10:30 h.

2.3. *Experimental procedure*

The incubation procedure and measurements of esterification of [1-^{14}C]oleate and its conversion to $^{14}CO_2$ were as in [15]. Acetoacetate and D-3-hydroxybutyrate were determined in neutralized $HClO_4$ extracts by enzymic methods [16]. The metabolite changes and $^{14}CO_2$ production between 20 and 60 min were calculated from plots of the values at 20, 40 and 60 min.

3. Results

3.1. *Effects of vasopressin on oxidation of [1-^{14}C]-oleate*

The addition of vasopressin (10 nM) to hepatocytes from fed rats in the presence of Ca^{2+} (2.4 mM)

Table 1
Comparison of effects of vasopressin, glucagon and dibutyryl cyclic-AMP on oxidation of [1-^{14}C]oleate to $^{14}CO_2$ in hepatocytes from fed rats

Additions	[1-^{14}C]Oleate oxidized to $^{14}CO_2$	
	+ $CaCl_2$	− $CaCl_2$
None	36 ± 2 (29)	33 ± 2 (19)
Vasopressin (10 nM)	60 ± 3 (26)[b]	40 ± 2 (19)[a]
Glucagon (10 nM)	25 ± 2 (12)[b]	24 ± 2 (11)[a]
Vasopressin (10 nM) + glucagon (10 nM)	38 ± 5 (8)	28 ± 3 (8)
Dibutyryl cyclic-AMP (10 μM)	31 ± 7 (3)	23 ± 2 (3)[a]
Vasopressin (10 nM) + dibutyryl cyclic-AMP (10 μM)	47 ± 7 (3)	26 ± 3 (3)[a]

[a] $P < 0.05$;
[b] $P < 0.0005$

Production of $^{14}CO_2$ is expressed as nmol . min^{-1} . g wet wt cells^{-1} and the results are mean values ± SEM with no. obs. in parentheses. Oleate was 1 mM and $CaCl_2$ was 2.4 mM

increased oxidation of [1-^{14}C]oleate to $^{14}CO_2$ by 66%. The increase was much less in the absence of added Ca^{2+} (table 1). In contrast, glucagon decreased [1-^{14}C]oleate oxidation to $^{14}CO_2$ by 31% and a similar decrease was observed in both the presence and the absence of Ca^{2+}. Such decreases were also found in the presence of dibutyryl cyclic-AMP (table 1). When both hormones were present at equimolar concentrations in the presence of Ca^{2+}, the rate of $^{14}CO_2$ production from [1-^{14}C]oleate was not significantly different from the control value without hormone (table 1). In the absence of Ca^{2+} the rate of oxidation of [1-^{14}C]oleate in the presence of both vasopressin and glucagon or dibutyryl cyclic-AMP was similar to that observed in the presence of dibutyryl cyclic-AMP alone.

3.2. *Effects of vasopressin on oleate oxidation and ketogenesis in the presence of lactate and pyruvate*

The rate of disposal of acetyl-CoA by the tricarboxylic acid cycle may be limited by the availability of oxaloacetate for citrate synthesis [17]. The K_m for pyruvate of pyruvate carboxylase (0.14 mM) is higher than the concentration of pyruvate in the liver [18,19]. Increased availability of lactate and pyruvate should therefore increase the production of oxaloacetate. The decreased rates of ketogenesis from oleate observed in the presence of vasopressin are concomitant with increased rates of lactate [8] and pyruvate (unpublished) production. The effects of vasopressin on ketogenesis from oleate and on oxidation of [1-^{14}C]oleate to $^{14}CO_2$ in the presence and absence of lactate (1 mM) and pyruvate (0.1 mM) were therefore investigated (table 2). Inclusion of lactate and pyruvate in the incubation medium increased $^{14}CO_2$ production from [1-^{14}C]oleate. Vasopressin increased oxidation in both the presence and absence of lactate and pyruvate (table 2). Maximal effects were observed in the presence of lactate and pyruvate and vasopressin (table 2). In both the presence of lactate and pyruvate, and the presence of vasopressin, the increases in $^{14}CO_2$ production were associated with decreased ketone body production. Again, maximal effects were observed when all three were present (table 2).

3.3. *Calcium dependency of the antiketogenic effect of vasopressin*

Vasopressin depressed ketone body formation from oleate in the presence of Ca^{2+} (table 3). This effect of vasopressin was decreased in the absence of

Table 2
Effects of lactate and pyruvate on [1-^{14}C]oleate oxidation in hepatocytes from fed rats

Additions	[1-^{14}C]oleate oxidized to $^{14}CO_2$		Ketone body production	
	+ $CaCl_2$	− $CaCl_2$	+ $CaCl_2$	− $CaCl_2$
None	31 ± 3 (8)	30 ± 3 (6)	713 ± 55 (12)	545 ± 58 (6)
Lactate (1 mM) + pyruvate (0.1 mM)	38 ± 4 (8)	36 ± 2 (6)	575 ± 33 (12)[a]	421 ± 52 (6)
Vasopressin (10 nM)	54 ± 5 (8)[c]	39 ± 4 (6)[a]	463 ± 50 (12)[b]	458 ± 50 (6)
Lactate (1 mM) + pyruvate (0.1 mM) + vasopressin (10 nM)	66 ± 7 (6)[c]	48 ± 4 (4)[b]	332 ± 35 (10)[c]	349 ± 80 (4)[a]

[a] $P < 0.05$;
[b] $P < 0.005$;
[c] $P < 0.0005$

Production of $^{14}CO_2$ is expressed as nmol . min . g wt cells^{-1} and the results are mean values ± SEM with no. obs. in parentheses. Oleate was 1 mM and $CaCl_2$ was 2.4 mM

Ca^{2+} (table 3). In marked contrast, esterification of [1-^{14}C]oleate was stimulated by vasopressin both in the presence and absence of calcium (table 2). This implies that the decreased rate of ketogenesis observed in the presence of vasopressin is not a consequence of an increased rate of esterification. However, in the same experiments increased oxidation of [1-^{14}C]-oleate in response to vasopressin was Ca^{2+}-dependent (table 3, see also table 1). Thus decreased ketogenesis from oleate may be related to increased oxidation of oleate.

4. Discussion

Vasopressin increases conversion of oleate into esterified products in hepatocytes from fed rats [8]. These studies demonstrate that, in addition to this extramitochondrial effect of vasopressin, the hormone has an intramitochondrial site of action, namely increased $^{14}CO_2$ production from [1-^{14}C]oleate.

Increased oxidation in response to vasopressin is observed in both the absence and in the presence of lactate and pyruvate. The increase in $^{14}CO_2$ produc-

Table 3
Effects of vasopressin on esterification and oxidation of [1-^{14}C]oleate and on ketogenesis in the presence or absence of Ca^{2+}

	[1-^{14}C]Oleate esterified		[1-^{14}C]Oleate oxidized to $^{14}CO_2$		Ketone body production	
	+ $CaCl_2$	− $CaCl_2$	+ $CaCl_2$	− $CaCl_2$	+ $CaCl_2$	− $CaCl_2$
None	187 ± 16 (9)	203 ± 25 (9)	36 ± 5 (9)	34 ± 4 (9)	662 ± 31 (26)	664 ± 40 (21)
Vasopressin (10 nM)	304 ± 27 (9)[b]	322 ± 42 (9)[a]	61 ± 7 (8)[a]	39 ± 4 (9)	413 ± 40 (27)[b]	580 ± 42 (19)

[a] $P < 0.01$;
[b] $P < 0.0005$

Metabolic rates expressed as nmol . min^{-1} . g wet wt cells^{-1} and the results are mean values ± SEM with no. obs. in parentheses. Oleate was 1 mM and $CaCl_2$ was 2.4 mM.

tion observed in the presence of lactate and pyruvate is presumably due to increased oxaloacetate availability. The additional change in $^{14}CO_2$ production produced by vasopressin in the presence of lactate and pyruvate is therefore likely to be due to a different mechanism. A possible action of vasopressin to decrease entry of long-chain acyl-CoA into the mitochondria might account for part of the antiketogenic effect. If such an action were secondary to a change in malonyl-CoA concentration, it could not account for the decreased ketogenesis elicited by vasopressin in the presence of lactate and pyruvate. Under these conditons the rate of lipogenesis (and presumably the malonyl-CoA concentration) was not further increased by vasopressin (control (3) 0.072 ± 0.006 μmol ^3H_2O . min^{-1} . g fresh wt $cells^{-1}$; plus vasopressin (3) 0.075 ± 0.004 μmol ^3H_2O . min^{-1} . g wet wt $cells^{-1}$). Moreover, 52% of the decrease in ketone body production observed on addition of vasopressin in the presence of lactate and pyruvate can be attributed to increased complete oxidation of oleate (table 2).

Both the increased oxidation to $^{14}CO_2$ of [1-^{14}C]-oleate in response to vasopressin, and the antiketogenic action of vasopressin were dependent on the addition of Ca^{2+} to the incubation medium. In contrast, esterification was stimulated by vasopressin both in the presence and absence of Ca^{2+}. This observation suggests that the intramitochondrial site of action of vasopressin plays the major role in the antiketogenic effect of the hormone when oleate is the exogenous substrate. Indeed, assuming the complete oxidation of oleoyl-CoA to acetyl-CoA, the data given in table 3 indicate that 45% of the decrease in ketone body production elicited by vasopressin can be accounted for by increased oxidation of oleate. The mechanism by which vasopressin increases $^{14}CO_2$ production from [1-^{14}C]oleate is not known. Such an increase might result from increased tricarboxylic acid cycle turnover. As it has been reported that certain enzymes of the tricarboxylic acid cycle are activated by Ca^{2+} [20,21] and vasopressin does not increase $^{14}CO_2$ production in the absence of Ca^{2+}, it is possible that the hormone exerts its effects by an increase in the mitochondrial Ca^{2+} concentration.

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