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Stimulation of hepatic lipogenesis and acetyl-coenzyme A carboxylase by vasopressin

Françoise ASSIMACOPOULOS-JEANNET,* Richard M. DENTON† and Bernard JEANRENAUD*
**Laboratoires de Recherches Métaboliques, 64 Avenue de la Roseraie, Département de Médecine, Geneva, Switzerland, and †Department of Biochemistry, University of Bristol Medical School, University Walk, Bristol BS8 1TD, U.K.*

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The effect of vasopressin on the short-term regulation of fatty acid synthesis was studied in isolated hepatocytes from rats fed *ad libitum*. Vasopressin stimulates fatty acid synthesis by 30–110%. This increase is comparable with that obtained with insulin. Angiotensin also stimulates fatty acid synthesis, whereas phenylephrine does not. The dose–response curve for vasopressin-stimulated lipogenesis is similar to the dose–response curve for glycogenolysis and release of lactate plus pyruvate. Vasopressin also stimulates acetyl-CoA carboxylase activity in a dose-dependent manner. Vasopressin does not relieve glucagon-inhibited lipogenesis, whereas insulin does. The action of vasopressin on hepatic lipogenesis is decreased, but not supressed, in Ca^{2+}-depleted hepatocytes. The results suggest that vasopressin acts on lipogenesis by increasing availability of lipogenic substrate (lactate + pyruvate) and by activating acetyl-CoA carboxylase.

Hepatic fatty acid synthesis is regulated by nutritional and hormonal factors. Long-term regulation of fatty acid synthesis involves changes in the concentration of lipogenic enzymes (Lakshmanan *et al.*, 1972; Craig & Porter, 1973; Nepokroeff *et al.*, 1974; Roncari & Murthy, 1975), mostly owing to increasing synthesis. Short-term regulation of lipogenesis may involve modification of substrate availability, of allosteric effectors, and activation or inactivation of pre-existing enzymes. Other data have shown that insulin and glucagon are able to modify acutely (respectively increase and decrease) fatty acid synthesis in isolated hepatocytes (Müller *et al.*, 1976; Geelen *et al.*, 1978; Witters *et al.*, 1979). These changes in fatty acid synthesis are correlated with changes in the activity of acetyl-CoA carboxylase (EC 6.4.1.2) (Geelen *et al.*, 1978; Witters *et al.*, 1979). On the other hand, little is known about lipogenic-substrate availability in the liver. Reports indicate that free glucose is a poor precursor of fatty acids, the major substrates being glycogen and lactate (Clark *et al.*, 1974).

Vasopressin used at supraphysiological concentrations has been shown to stimulate glycogen phosphorylase and glycogenolysis (Hems *et al.*, 1975; Keppens & De Wulf, 1975). These actions are independent of cyclic AMP and require extracellular Ca^{2+} (Stubbs *et al.*, 1976; Keppens *et al.*, 1977). Vasopressin also increases the active form of pyruvate dehydrogenase in the perfused liver of the rat by an unknown mechanism (Hems *et al.*, 1978). In the liver, as in other tissues, pyruvate dehydrogenase provides acetyl-CoA, which is oxidized or converted into fatty acids and cholesterol. Therefore a stimulatory effect of vasopressin on hepatic lipogenesis would be a logical consequence of its action on pyruvate dehydrogenase.

On the basis of this rationale, the action of vasopressin was investigated in the present study, in isolated hepatocytes from fed rats. The results show that vasopressin stimulates hepatic lipogenesis in a dose-dependent manner. This hormone also stimulates acetyl-CoA carboxylase, which is considered one of the rate-limiting steps in fatty acid synthesis (Chang *et al.*, 1967).

Experimental

Animals

Male albino rats (10–11 weeks old) derived from a Wistar strain bred in these laboratories were used. They weighed between 220 and 270g and were fed *ad libitum* with standard laboratory chow.

Chemicals

Collagenase, [arginine]vasopressin (grade VI) and [isoleucine]angiotensin II were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All enzymes and coenzymes were obtained from Boehringer (Mannheim, Germany). $NaH^{14}CO_3$ and

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^3H_2O were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Pig insulin (10 times recrystallized) was from Novo Research Institute (Copenhagen, Denmark). Bovine albumin fraction V from Sigma was defatted by the method of Chen (1967), and dialysed against water.

Methods

Hepatocytes were prepared between 09:00 and 10:00h by collagenase digestion (Le Cam *et al.*, 1976; Le Cam & Freychet, 1977). Viability of cells was judged by their ability to exclude Trypan Blue and was 92–96%. After washing, the cells were preincubated for 30min in a Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) containing 3% (w/v) defatted bovine albumin and 10mM-glucose. Cells were then centrifuged and resuspended in the same buffer. Portions (2ml) of cell suspension (50mg wet wt./ml) containing ^3H_2O (0.1mCi/ml) were distributed in a 25ml plastic Erlenmeyer flask with or without hormones. Incubations were carried out at 37°C in a rotating incubator. The cells were gassed with O_2/CO_2 (19:1) for 2min every 30min. The total rate of lipogenesis was estimated by measuring the incorporation of ^3H from ^3H_2O into saponifiable lipids as described for adipose tissue (Jungas, 1968) and liver (Windmueller & Spaeth, 1966). At the end of the incubation, cells were rapidly separated from the medium and either extracted for determination of labelled lipid or frozen in liquid N_2 for further determination of acetyl-CoA carboxylase activity. Total lipids were extracted in chloroform/methanol (Folch *et al.*, 1957). The chloroform phase was dried and saponified in ethanolic 0.5M-KOH. The unsaponifiable fraction was discarded, and saponifiable lipids were extracted with light petroleum (b.p. 30–45%) after acidification of the solution. The extract was dried and counted for radioactivity. The incubation medium was deproteinized with 0.15M-$ZnSO_4$ and 0.075M-$Ba(OH)_2$. Glucose, lactate and pyruvate were measured as described by Bergmeyer (1974).

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. For the determination of acetyl-CoA carboxylase, the cell pellet was homogenized in a glass tissue grinder with 12 strokes of a motor-driven Teflon pestle, in 1vol. of 100mM-potassium phosphate/2mM-EDTA/20mM-β-mercaptoethanol (pH7.3). Acetyl-CoA carboxylase activity was measured in the supernatant after centrifugation at 25000*g* for 20min by measuring the incorporation of [^{14}C]bicarbonate into acid-stable material at 30°C for 2min, by techniques published elsewhere (Majerus *et al.*, 1968; Halestrap & Denton, 1973). The reaction was initiated by adding 50μl of supernatant to 500μl of 50mM-Tris/HCl, pH7.4, containing 10mM-$MgSO_4$, 0.5mM-EDTA, albumin (1mg/ml), 1mM-ATP, 1mM-dithiothreitol and 15mM-$KH^{14}CO_3$ (0.5Ci/mol). A blank for acetyl-CoA-independent $^{14}CO_2$ fixation was run with each sample. The enzyme activity was measured in the 25000*g* supernatant in the absence of added citrate ('initial' activity) and also after 10min of incubation of the supernatant with 10mM-potassium citrate ('+citrate' activity). The assay was linear with time and protein concentration. Results for acetyl-CoA carboxylase were expressed as munits/min per mg of protein in the 25000*g* supernatant, and for 'initial' activity as a percentage of '+citrate' activity. One unit represents 1μmol of substrate converted/min.

Every experiment was repeated three to five times. The effects of vasopressin on fatty acid synthesis were consistently observed and varied between 30 and 110% increase over the control. Since basal lipogenesis varied in our conditions from 10 to 25μg-atoms of ^3H incorporated into fatty acids/h per g, one representative experiment is shown. Statistics were performed by the Student's *t* test.

Results

Stimulation of hepatic lipogenesis by vasopressin and angiotensin

Hepatocytes from fed rats incubated with 10mM-glucose and ^3H_2O show rates of incorporation of ^3H into triacylglycerol fatty acids of 10–25μg-atoms of ^3H/h per g. These values are equal to or slightly lower than values reported *in vivo* (Stansbie *et al.*, 1976) or in similar conditions in perfused rat liver (Brunengraber *et al.*, 1973) or in isolated hepatocytes (Clark *et al.*, 1974). As described previously by others (Witters *et al.*, 1979; Geelen *et al.*, 1978), insulin increases hepatic lipogenesis (Fig. 1). Vaso-

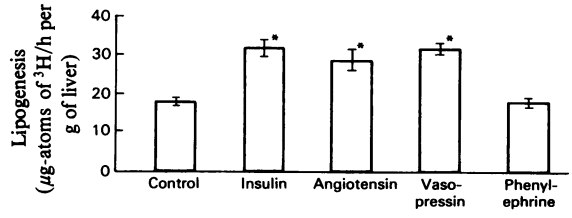

Fig. 1. *Effect of insulin, angiotensin, vasopressin and phenylephrine on lipogenesis*

Hepatocytes from fed rats were incubated for 60min with 10mM-glucose, ^3H_2O (0.1mCi/ml) and hormones (concns.: insulin, 13.4nM; angiotensin, 20nM; vasopressin, 10.8nM; phenylephrine, 10μM). Results are means ±S.E.M. for triplicate incubations in one representative experiment, expressed as μg-atoms of ^3H incorporated into saponifiable fatty acid/h per g of liver. *$P < 0.01$ compared with control.

pressin also stimulates fatty acid synthesis. The effects of angiotensin and phenylephrine, which have the same Ca^{2+}-dependent action as vasopressin on glycogen phosphorylase and glycogenolysis (Keppens *et al.*, 1977), were also tested. Angiotensin shows a stimulation of lipogenesis similar to that of vasopressin, whereas phenylephrine does not modify this parameter.

Although not shown in Fig. 1, the stimulatory action of vasopressin is measurable 20 min after the addition of the hormone.

The dose–response of vasopressin-stimulated lipogenesis is parallel to that observed for glycogenolysis and glycolysis (Table 1). However, half-maximal stimulation of lipogenesis occurs at about 0.5 nM, whereas half-maximal stimulation of glycogenolysis and release of lactate plus pyruvate is observed at lower concentrations.

As shown, release of lactate and pyruvate is markedly increased by vasopressin (Table 1). These two compounds are much better lipogenic substrates than is glucose (Clark *et al.*, 1974). One of the mechanisms of action of vasopressin could be to increase the availability of lactate and pyruvate. In addition, since this hormone increases the activity of pyruvate dehydrogenase (Hems *et al.*, 1978), these two actions would lead to increased production of acetyl-CoA and increased lipogenesis. To test this possibility we incubated hepatocytes with two concentrations of lactate and pyruvate (in order to mimic the effect of vasopressin on lactate and pyruvate release). Table 2 shows that when lactate and pyruvate concentration is increased in the incubation medium, basal lipogenesis increases. At the same time, vasopressin and insulin no longer stimulate lipogenesis.

Effect of vasopressin on the activity of acetyl-CoA carboxylase

The action of vasopressin on acetyl-CoA carboxylase was measured in hepatocytes incubated for 20 min in the presence of the hormone. Vasopressin increases the 'initial' activity of the enzyme and slightly, but not always significantly, the activity measured after preincubation of the supernatant with 10 mM-citrate for 10 min (Table 3). The dose–response of vasopressin stimulation of acetyl-CoA carboxylase measured at 20 min is identical with the dose–response of vasopressin-stimulated lipogenesis, i.e. the percentage stimulation by a given concentration of hormone was the same for the two parameters (compare Tables 1 and 3). No effect of vasopressin on acetyl-CoA carboxylase was detected 1 min after addition of the hormone, but a significant increase was measured after 10 min (results not shown).

Effect of vasopressin and insulin on glucagon-inhibited lipogenesis

Hepatocytes incubated with glucagon (0.5 nM) show a 65% inhibition of the incorporation of ^3H into saponifiable fatty acids (Fig. 2).

Insulin, which antagonizes many of the actions of glucagon on hepatic metabolism, is able to decrease

Table 2. *Effect of insulin and vasopressin on lipogenesis in the presence of lactate and pyruvate*

Hepatocytes from fed rats were incubated for 60 min with substrates at the concentration mentioned and ^3H_2O (0.1 mCi/ml). Results are means ± S.E.M. for triplicate incubations in one representative experiment. [a] $P < 0.0005$ compared with control.

Addition of hormone	Lipogenesis (μg-atoms of ^3H/h per g of liver): Lactate (2 mM) + pyruvate (0.2 mM)	Lactate (10 mM) + pyruvate (1 mM)
None	35.2 ± 0.3	49.1 ± 1.7
Vasopressin (10.8 nM)	43.7 ± 1.1[a]	51.1 ± 1.2
Insulin (6.7 nM)	47.3 ± 1.4[a]	52.2 ± 1.8

Table 1. *Dose–response of the effect of vasopressin on hepatic lipogenesis and release of glucose, lactate and pyruvate*

Hepatocytes from fed rats were incubated for 60 min with 10 mM-glucose, ^3H_2O (0.1 mCi/ml), and hormone at the indicated concentration. Results are means ± S.E.M. for triplicate incubations in one representative experiment. [a] $P < 0.05$, [b] $P < 0.005$, [c] $P < 0.001$ compared with control.

Addition	Lipogenesis (μg-atoms of ^3H/h per g of liver)	Glucose release (μmol/h per g of liver)	Lactate + pyruvate release (μmol/h per g of liver)
None	11.4 ± 0.8	6.2 ± 1.2	8.1 ± 0.9
Vasopressin (0.27 nM)	10.1 ± 0.5	17.1 ± 1.5[b]	18.6 ± 1.5[b]
Vasopressin (0.54 nM)	17.6 ± 2.3	24.2 ± 0.6[c]	21.3 ± 0.5[c]
Vasopressin (1.35 nM)	21.0 ± 0.8[b]	25.0 ± 2.4[b]	22.4 ± 1.5[b]
Vasopressin (2.70 nM)	21.0 ± 0.4[c]	31.4 ± 1.9[c]	24.6 ± 1.3[c]
Vasopressin (10.80 nM)	20.4 ± 0.7[b]	32.9 ± 2.1[c]	24.5 ± 1.8[b]
Insulin (6.7 nM)	19.7 ± 0.8[b]	1.1 ± 1.1[a]	7.4 ± 2.3

or to suppress the inhibition of lipogenesis produced by glucagon. Fig. 2 shows that vasopressin does not significantly modify glucagon-inhibited lipogenesis.

Table 3. *Dose–response of the effects of vasopressin on acetyl-CoA carboxylase*

Hepatocytes from fed rats were incubated for 20 min with 10 mM-glucose and vasopressin at the indicated concentration. Acetyl-CoA carboxylase was measured as described in the Experimental section. Results are means ± S.E.M. for triplicate incubations in one representative experiment. $^{a}P < 0.025$, $^{b}P < 0.01$ compared with control.

Addition of vasopressin	Acetyl-CoA carboxylase (munits/min per mg of protein) 'initial'	'+citrate'	Active enzyme (% of total)
0	1.80 ± 0.12	10.20 ± 0.42	18 ± 1
0.27 nM	2.02 ± 0.36	10.56 ± 0.64	19 ± 2
1.35 nM	3.49 ± 0.54[a]	12.31 ± 0.75	28 ± 3[a]
2.70 nM	3.56 ± 0.31[b]	12.15 ± 0.20[b]	29 ± 3[a]
10.80 nM	4.32 ± 0.80[a]	12.32 ± 0.66	34 ± 4[b]

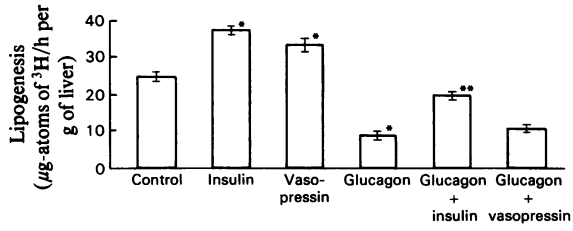

Fig. 2. *Effect of vasopressin and insulin on glucagon-inhibited lipogenesis*

Hepatocytes from fed rats were incubated for 60 min with 10 mM-glucose, $^{3}H_2O$ (0.1 mCi/ml) and hormones (concns.: insulin, 6.7 nM; vasopressin, 10.8 nM; glucagon, 0.5 nM). Results are means ± S.E.M. for triplicate incubations in one representative experiment, expressed as μg-atoms of ^{3}H incorporated into saponifiable fatty acid/h per g of liver. $^{*}P < 0.01$ compared with control; $^{**}P < 0.01$ compared with glucagon alone.

Effect of vasopressin on lipogenesis in Ca^{2+}-depleted hepatocytes

Since vasopressin is known to require Ca^{2+} to stimulate glycogen phosphorylase and glycogenolysis (Stubbs *et al.*, 1976; Keppens *et al.*, 1977), lipogenesis was measured in Ca^{2+}-deprived hepatocytes with and without vasopressin. Cells were washed, preincubated and incubated in Ca^{2+}-free Krebs–Ringer bicarbonate buffer without added EDTA. The Ca^{2+} depletion was sufficient to suppress the action of vasopressin on glucose release. Under these conditions, however, vasopressin still retained some stimulatory effect on lipogenesis (Table 4), although its action was decreased compared with that observed in cells incubated with normal Ca^{2+} concentration. Similar results were obtained when hepatocytes were washed, preincubated and incubated in Ca^{2+}-free Krebs–Ringer bicarbonate buffer containing 0.5 mM-EDTA (results not shown).

Discussion

This study indicates that vasopressin and angiotensin (but not phenylephrine) stimulate hepatic synthesis of fatty acids. The dose–response of vasopressin-stimulated lipogenesis is in the range of its action on other metabolic parameters such as glycogenolysis (Table 1) and glycogen phosphorylase (Keppens & De Wulf, 1975).

Vasopressin may act on hepatic lipogenesis at different levels. The hormone increases glycolysis, thereby providing lipogenic substrates. One should mention that production of lactate and pyruvate is only a very rough estimation of glycolysis and certainly underestimates the effect of vasopressin on this parameter, since the hormone also increases the activity of pyruvate dehydrogenase (Hems *et al.*, 1978).

Previous studies have reported that vasopressin does not modify lipogenesis in the perfused rat liver (Kirk & Hems, 1979) and even inhibits this process in the perfused mouse liver (Ma & Hems, 1975). Those studies have, however, been carried out with

Table 4. *Effect of vasopressin and insulin on lipogenesis in Ca^{2+}-depleted hepatocytes*

Hepatocytes from fed rats were washed, preincubated and incubated either in normal Krebs–Ringer bicarbonate buffer medium or in Ca^{2+}-depleted Krebs–Ringer bicarbonate buffer. They were incubated for 60 min with 10 mM-glucose, $^{3}H_2O$ (0.1 mCi/ml), and hormone at the indicated concentration. Results are means ± S.E.M. for triplicate incubations in one representative experiment. $^{a}P < 0.001$ compared with control.

	Addition	Lipogenesis (μg-atoms of ^{3}H/h per g of liver)	Glucose release (μmol/h per g of liver)
Normal hepatocytes	None	15.8 ± 0.7	9.7 ± 1.0
	Vasopressin (10.8 nM)	22.8 ± 0.4[a]	25.1 ± 2.2[a]
Ca^{2+}-depleted hepatocytes	None	14.0 ± 0.3	11.1 ± 2.5
	Vasopressin (10.8 nM)	17.5 ± 0.2[a]	13.6 ± 1.8

high lactate concentrations (4 or 16mM). Under these conditions, lipogenesis may be maximally stimulated. Data of the present study (Table 2) are in keeping with such an interpretation, since, when lactate concentration in the medium is increased, basal lipogenesis is increased, and the stimulatory effect of vasopressin as well as that of insulin is lost.

Vasopressin also affects lipogenesis at the level of acetyl-CoA carboxylase, since, as shown by the present study, the hormone does increase the 'initial' activity of acetyl-CoA carboxylase and also slightly increases the activity of the enzyme after pre-incubation with 10mM-citrate. The basal values of percentage of active acetyl-CoA carboxylase measured in the present study are much lower than those measured *in vitro* in fed rats (43% active) by Stansbie *et al.* (1976), and in perfused mouse liver (56% active) by Hems (1977). They are slightly lower than those measured by Witters *et al.* (1979) (30% active) in isolated hepatocytes from starved/refed rats incubated with 10mM-glucose. Acetyl-CoA carboxylase is known to be modified by allosteric factors as well as by covalent modifications (Lowenstein, 1967; Lee & Kim, 1977; Brownsey *et al.*, 1979, 1981; Brownsey & Denton, 1979). These mechanisms may be complementary (Witters *et al.*, 1979; Carlson & Kim, 1974). The main allosteric effectors of acetyl-CoA carboxylase are citrate and fatty acyl-CoA, which affect the protomer–polymer equilibrium of the enzyme. In our experiments, measurement of citrate shows no change in intact cells with or without vasopressin (results not shown). It has been reported that vasopressin promotes esterification of fatty acyl-CoA (Williamson *et al.*, 1980). By this action the hormone may relieve an inhibition of the enzyme, although one does not expect fatty acyl-CoA concentration to be high in hepatocytes from fed animals incubated without fatty acids.

Insulin increases the initial activity of acetyl-CoA carboxylase in fat-cells (Halestrap & Denton, 1974; Brownsey & Denton, 1979), and this may be brought about through increased phosphorylation (Brownsey *et al.*, 1981). Stimulation of acetyl-CoA carboxylase in liver cells exposed to insulin has also been described (Witters *et al.*, 1979; Geelen *et al.*, 1978). However, the action of insulin on liver cells is only evident in homogenates prepared and kept at room temperature, whereas the inhibition produced by glucagon is still measurable after cooling of the cells or the homogenate. Since the activation of the enzyme observed in the presence of vasopressin in the present studies is measured in cells that have been frozen in liquid N_2, the mechanism may be different from the increase in liver cells promoted by insulin. At the present time, no hypothesis can be made about the mechanism by which vasopressin stimulates acetyl-CoA carboxylase.

Ca^{2+} depletion, which totally prevents the action of vasopressin on glycogen phosphorylase and on glycogenolysis (Stubbs *et al.*, 1976), does not prevent its stimulatory action on phosphatidylinositol breakdown and resynthesis (Billah & Michell, 1979). In the present study, the action of vasopressin on hepatic lipogenesis is markedly decreased by omission of Ca^{2+} (but not totally suppressed), whereas the action on glucose release as reflected by output of glucose and lactate plus pyruvate is suppressed. This suggests that the increase in lipogenesis is not simply the consequence of the increased glycogenolysis and that some step insensitive or less sensitive to Ca^{2+} may be involved in the action of vasopressin.

The antagonistic action of vasopressin on glucagon-stimulated ketogenesis has been reported (Williamson *et al.*, 1980). In our experiments, vasopressin does not counteract the inhibitory action of glucagon on lipogenesis and on acetyl-CoA carboxylase (F. Assimacopoulos-Jeannet, unpublished work), whereas insulin does. The action of vasopressin on hepatic lipogenesis may not necessarily have physiological implications. Thus the concentrations of the hormone required to elicit effects *in vitro* are above the basal ones reported in blood (Forsling, 1976). In spite of this, vasopressin constitutes an interesting tool with which to study the respective roles of substrate availability and enzyme activity in the regulation of hepatic lipogenesis.

To sum up, data from the literature and from the present study show that vasopressin stimulates liver lipogenesis by: (*a*) increasing glycolysis, thereby providing lipogenic substrates; (*b*) increasing pyruvate dehydrogenase activity, providing acetyl-CoA (Hems *et al.*, 1978); (*c*) increasing acetyl-CoA carboxylase activity and thereby increasing fatty acid synthesis.

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