Inhibition of lipogenesis by vasopressin and angiotensin II in glycogen-depleted hepatocytes

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Vasopressin and angiotensin II inhibited lipogenesis (measured with $^3$H$_2$O) in hepatocytes from fed rats. Inhibition was also observed with hepatocytes from fed rats which had been depleted of glycogen in vitro and incubated with lactate + pyruvate (5 mM + 0.5 mM) as substrates. The inhibitory actions of the hormones are therefore independent of hormone-mediated changes in glycogenolytic or glycolytic flux from glycogen, and thus the site(s) of hormone action must be subsequent to the formation of lactate. (-)Hydroxycitrate, a specific inhibitor of ATP-citrate lyase, decreased lipogenesis in hepatocytes from fed rats incubated with lactate + pyruvate by approx. 51% but had little effect on lipogenesis in glycogen-depleted hepatocytes similarly incubated. There was parallel inhibition of incorporation of $^{14}$C from [U-$^{14}$C]lactate into fatty acid and lipogenesis as measured with $^3$H$_2$O in each case. Thus depletion of glycogen, or conceivably the process of glycogen-depletion (incubation with dibutyryl cyclic AMP) causes a change in the rate-determining step(s) for lipogenesis from lactate. Vasopressin and angiotensin II also decreased lipogenesis and incorporation of $^{14}$C into fatty acids in glycogen-depleted hepatocytes provided with [U-$^{14}$C]proline as opposed to [U-$^{14}$C]-lactate. However, proline-stimulated lipogenesis was inhibited by (-)hydroxycitrate, and proline-stimulated lipogenesis and incorporation of $^{14}$C from [U-$^{14}$C]-proline were not decreased in parallel by this inhibitor (inhibition of 52% and 85% respectively). It is inferred that lactate and proline stimulate lipogenesis by different mechanisms and incorporation of $^{14}$C from [U-$^{14}$C]proline and [U-$^{14}$C]lactate into fatty acid occurs via different routes. (-)Hydroxycitrate diminished the inhibitory effects of the hormones in the presence of either lactate or proline, suggesting that flux through ATP-citrate lyase is important for the hormone response.
Vasopressin, angiotensin II, adrenaline, and noradrenaline have a number of similar actions on liver carbohydrate metabolism, including stimulation of glycogenolysis and gluconeogenesis (reviewed by Williamson et al., 1981). The hormones also have similar actions to increase the oxidation to CO₂ of short- and long-chain-length fatty acids (Sugden et al., 1980a,b; Watts & Sugden, 1983; Palmer et al., 1983). The effects of the four hormones on fat synthesis are less well-defined. It is generally agreed that the catecholamines inhibit hepatic fatty acid synthesis (Ly & Kim, 1981; Ma et al., 1977), but vasopressin and angiotensin II have been variously reported to inhibit, stimulate, or have no effect on hepatic lipogenesis (Ma & Hems, 1975; Ma et al., 1977; Kirk & Hems, 1979; Williamson et al., 1980; Assimacopoulos-Jeannet et al., 1981).

In the liver, lactate is a good lipogenic precursor (Salmon et al., 1978) and stimulatory effects of vasopressin and angiotensin II on lipogenesis (in hepatocytes from fed rats) have been suggested to result possibly from increased provision of lactate, secondary to increased glycolysis (Assimacopoulos-Jeannet et al., 1981). In order to establish whether any change in lipogenesis is a consequence of hormonal modulation of glycolysis from glycogen, the present work examined the effects of vasopressin and angiotensin II on lipogenesis in hepatocytes from fed rats depleted of glycogen in vitro. To attempt to define the site of action of the hormones, glycogen-depleted hepatocytes were incubated with either lactate and pyruvate or proline as substrates. Whereas the utilization of lactate carbon for lipogenesis proceeds via its conversion by the pyruvate dehydrogenase complex to mitochondrial acetyl-CoA and thence to citrate, proline can be metabolized to citrate (Hensgens et al., 1978) via alternative routes, namely by conversion to 2-oxoglutarate, and then via the reactions of the tricarboxylic acid cycle in the mitochondria, or by a cytoplasmic pathway involving cytoplasmic NADP⁺-isocitrate dehydrogenase (EC 1.1.1.42) (Madsen et al., 1964a,b; D’Adamo & Haft, 1965; Scholz & Evans, 1977). To eliminate the possible conversion of proline to citrate via lactate (via glutamate, oxaloacetate, and phosphoenolpyruvate), experiments were also carried out in the presence of 3-mercaptopicolinate (3-MPA) which inhibits liver phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (DiTullio et al., 1974). Use was also made of a specific inhibitor of ATP-citrate lyase (EC 4.1.3.8), (-)hydroxycitrate [(-)HC](Watson et al., 1969). (-)HC would be expected to inhibit lipogenesis where citrate is a major contributor to the cytoplasmic acetyl-CoA pool, and so use of this inhibitor should allow distinctions to be made between possible effects of the hormones proximal or distal to ATP-citrate lyase in the lipogenic pathway.

Materials and Methods

Sources of materials were as given in Sugden and Watts (1983). Female albino Wistar rats (170-200 g) were subjected to a 12 h-light/12 h-dark cycle, the light period starting at 0800. Rats were allowed free access to standard rodent diet.

Hepatocyte preparation was commenced between 0830 and 0930. Isolated hepatocytes were prepared as described by Berry & Friend (1969), modified as in Krebs et al. (1974). Glucose was not present
LIPOGENESIS INHIBITION IN HEPATOCYTES

in the perfusion medium. Hepatocytes were suspended in Krebs and Henseleit (1932) bicarbonate buffer, pH 7.4. More than 90% of the cells excluded Trypan Blue. Hepatocytes prepared from fed rats were depleted of glycogen in vitro by incubation for 1 h with dibutylryl cyclic AMP followed by centrifugation and extensive washing to remove dibutylryl cyclic AMP, glucose, and lactate as described by Williamson and Whitelaw (1978). This procedure removes 90-95% of the cell glycogen (Williamson & Whitelaw, 1978). Glycogen depletion decreased cell viability assessed by Trypan Blue exclusion by about 10%.

The incubation procedure for measurements of lipogenesis was as described by Agius and Vaartjes (1982). The media contained 5 mM glucose. 3-MPA and (-)-HC were added as the Na + salts, pH 7.4. Rates of fatty acid synthesis and incorporation of 14C from [14C]-substrates were linear for up to 90 min with hepatocytes from fed rats and for up to 120 min with glycogen-depleted hepatocytes. At the end of the incubation period the flask contents were deproteinized with HClO4 and were saponified, and the saponified fatty acids were extracted as described by Stansbie et al. (1976).

Results are expressed as means ± S.E.M. with the number of hepatocyte preparations given in parentheses. Statistical significance of differences was assessed with Student's paired t-test.

Results

Hepatocytes from fed rats

Lipogenesis from endogenous substrates (measured by the incorporation into saponifiable fatty acid of 3H from 3H2O) was decreased by 15 ± 4% by angiotensin II and by 31 ± 5% by vasopressin (Table I). Lipogenesis was not significantly increased by addition of lactate + pyruvate (5 mM + 0.5 mM respectively) nor did the addition of these substrates affect the ability of vasopressin and angiotensin II to inhibit lipogenesis (inhibition of 17 ± 4% and 23 ± 2% by angiotensin II and vasopressin respectively). The inhibition of incorporation of 3H from 3H2O was paralleled by inhibition of incorporation of 14C from [U-14C]lactate into fatty acid (shown in square brackets in Table I).

Rates of lipogenesis were significantly decreased by (-)-HC in the absence or presence of the hormones and/or lactate + pyruvate (inhibition of 50-60%) (Table I). Others have observed that (-)-HC at this concentration inhibits lipogenesis in hepatocytes from fed, but not starved, rats (Brunengraber et al., 1978). The hormones did not significantly inhibit lipogenesis when (-)-HC was present (Table I). The changes in lipogenic rates (measured with 3H2O) in the presence of lactate + pyruvate were paralleled by changes in rates of incorporation of 14C from [U-14C]lactate (shown in square brackets in Table I) (20 ± 5% versus 44 ± 4% inhibition, P < 0.005). Inhibitory effects of angiotensin II on 14C-incorporation were not statistically significant in the presence of (-)-HC.

Glycogen-depleted hepatocytes

When no substrate was provided, glycogen-depleted hepatocytes from fed rats showed low basal rates of lipogenesis. The decreased
Table 1. Effects of vasopressin and angiotensin II on lipogenesis in hepatocytes from fed rats

Untreated or glycogen-depleted hepatocytes were incubated in the absence or presence of lactate (5 mM, 0.1 μCi/ml) + pyruvate (0.5 mM) and (-)HC (2 mM). Lipogenesis is given as μmol 3H2O incorporated into saponifiable fatty acid/h per g wet wt. cells. Incorporation of 14C from [U-14C]lactate is also shown (in square brackets) and is expressed as μg atoms of 14C incorporated into saponifiable fatty acid/h per g wet wt. of cells.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Control</th>
<th>+ Angiotensin II (10 nM)</th>
<th>Control</th>
<th>+ Vasopressin (10 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14.7 ± 0.6 (5)</td>
<td>12.5 ± 0.5 (5)*</td>
<td>14.9 ± 1.4 (4)</td>
<td>10.4 ± 1.6 (4)***</td>
</tr>
<tr>
<td>(-)Hydroxycitrinate</td>
<td>6.3 ± 0.3 (4)</td>
<td>5.8 ± 0.6 (4)</td>
<td>6.4 ± 0.2 (4)</td>
<td>6.0 ± 0.5 (4)</td>
</tr>
<tr>
<td>Lactate + pyruvate</td>
<td>16.9 ± 1.9 (6)</td>
<td>14.0 ± 1.9 (6)**</td>
<td>17.6 ± 1.7 (6)</td>
<td>13.6 ± 1.6 (6)****</td>
</tr>
<tr>
<td></td>
<td>[19.5 ± 2.1 (6)]</td>
<td>[15.3 ± 2.1 (6)]***</td>
<td>[21.5 ± 2.8 (5)]</td>
<td>[15.4 ± 2.5 (5)]***</td>
</tr>
<tr>
<td>Lactate + pyruvate + (-)hydroxycitrinate</td>
<td>7.1 ± 0.8 (6)</td>
<td>7.2 ± 0.7 (6)</td>
<td>8.0 ± 0.6 (5)</td>
<td>7.3 ± 0.8 (5)</td>
</tr>
<tr>
<td></td>
<td>[8.6 ± 1.6 (6)]</td>
<td>[7.7 ± 0.9 (6)]</td>
<td>[9.3 ± 1.1 (4)]</td>
<td>[8.0 ± 1.3 (4)]**</td>
</tr>
<tr>
<td>Glycogen-depleted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3.4 ± 0.4 (6)</td>
<td>3.0 ± 0.2 (6)</td>
<td>3.1 ± 0.6 (6)</td>
<td>2.1 ± 0.4 (6)</td>
</tr>
<tr>
<td>(-)Hydroxycitrinate</td>
<td>4.2 ± 0.6 (7)</td>
<td>[3.7 ± 0.4 (7)]</td>
<td>3.7 ± 0.8 (5)</td>
<td>[2.8 ± 0.4 (5)]</td>
</tr>
<tr>
<td>Lactate + pyruvate</td>
<td>9.0 ± 1.6 (6)</td>
<td>6.0 ± 1.2 (6)*</td>
<td>7.4 ± 1.2 (8)</td>
<td>4.3 ± 0.9 (8)****</td>
</tr>
<tr>
<td></td>
<td>[6.2 ± 1.1 (7)]</td>
<td>[4.2 ± 0.9 (7)]**</td>
<td>[6.0 ± 1.0 (8)]</td>
<td>[3.4 ± 0.7 (8)]****</td>
</tr>
<tr>
<td>Lactate + pyruvate + (-)hydroxycitrinate</td>
<td>7.5 ± 0.5 (7)</td>
<td>7.5 ± 0.6 (7)</td>
<td>6.1 ± 0.8 (6)</td>
<td>4.5 ± 0.6 (6)*</td>
</tr>
<tr>
<td></td>
<td>[5.2 ± 0.7 (6)]</td>
<td>[5.0 ± 1.0 (6)]</td>
<td>[5.1 ± 0.6 (6)]</td>
<td>[4.1 ± 0.6 (6)]</td>
</tr>
</tbody>
</table>

Values in the presence of the hormones that are significantly different from those in their absence are indicated: * P < 0.05; ** P < 0.02, *** P < 0.005; **** P < 0.001.
Table 2. Lipogenesis in glycogen-depleted hepatocytes in the presence of proline

Glycogen-depleted hepatocytes were incubated with proline (5 mM, 0.1 μCi/ml). Lipogenesis is given as umol of \(^{3}\)H\(_2\)O incorporated into saponifiable fatty acid/h per g wet wt. cells. Incorporation of \(^{14}\)C from [\(^{14}\)C]proline is also shown (in square brackets) and is expressed as μg atoms of \(^{14}\)C incorporated into saponifiable fatty acid/h per g wet wt. of cells.

<table>
<thead>
<tr>
<th>Hormone addition</th>
<th>Control</th>
<th>+ (-)Hydroxycitrate (2 mM)</th>
<th>+ 3-MPA (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12.4 ± 0.9 (4)</td>
<td>6.0 ± 0.2 (5)</td>
<td>10.9 ± 1.0 (5)</td>
</tr>
<tr>
<td>[11.2 ± 1.9 (4)]</td>
<td>[0.9 ± 0.2 (4)]</td>
<td>[9.5 ± 1.2 (4)]</td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td>6.9 ± 1.1 (4)***</td>
<td>4.4 ± 0.1 (5)***</td>
<td>8.0 ± 0.6 (5)*</td>
</tr>
<tr>
<td>(10 nM)</td>
<td>[5.1 ± 1.7 (4)]**</td>
<td>[0.5 ± 0.1 (4)]*</td>
<td>[5.6 ± 1.0 (4)]*</td>
</tr>
<tr>
<td>None</td>
<td>12.3 ± 1.2 (6)</td>
<td>6.0 ± 0.4 (6)</td>
<td>12.2 ± 1.3 (6)</td>
</tr>
<tr>
<td>[10.5 ± 1.4 (6)]</td>
<td>[0.6 ± 0.04 (6)]</td>
<td>[8.9 ± 1.4 (6)]</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>10.1 ± 1.1 (6)****</td>
<td>5.6 ± 0.5 (6)</td>
<td>9.6 ± 1.2 (6)****</td>
</tr>
<tr>
<td>(10 nM)</td>
<td>[7.9 ± 1.3 (6)]****</td>
<td>[0.4 ± 0.04 (6)]****</td>
<td>[5.6 ± 1.0 (6)]****</td>
</tr>
</tbody>
</table>

Statistically significant effects of the hormones are indicated by: * P < 0.05; ** P < 0.01; *** P < 0.005; **** P < 0.001.

Rates of lipogenesis are the result of glycogen depletion (since glycogen is a good lipogenic substrate) and possibly acute changes in the activities of a number of lipogenic enzymes (see Discussion and also Sugden & Williamson, 1981). Rates of lipogenesis were increased by the addition of lactate + pyruvate by 139-165% and incorporation of \(^{14}\)C into saponifiable fatty acid from [\(^{14}\)C]lactate was observed (Table 1). Rates of lipogenesis in the presence of lactate + pyruvate were inhibited by vasopressin and angiotensin II by 44 ± 3% and 25 ± 6% respectively (Table 1). The incorporation of \(^{14}\)C from [\(^{14}\)C]-lactate was inhibited by vasopressin or angiotensin II to a similar extent (Table 1).

The rates of lipogenesis in hepatocytes provided with lactate + pyruvate and \(^{14}\)C-incorporation from [\(^{14}\)C]lactate were not significantly inhibited by (-)HC in the absence or presence of the hormones. (-)HC decreased the inhibitory effects of vasopressin on lipogenesis (25 ± 6% versus 44 ± 3% inhibition, \(P < 0.01\)) and \(^{14}\)C- incorporation from [\(^{14}\)C]lactate (20 ± 5% versus 44 ± 4% inhibition, \(P < 0.005\)). Inhibitory effects of angiotensin II were not statistically significant in the presence of (-)HC.

The addition of proline, like that of lactate + pyruvate, stimulated lipogenesis in glycogen-depleted hepatocytes (by 261-300%) and incorporation of \(^{14}\)C from [\(^{14}\)C]proline was observed (Table 2). Vasopressin inhibited proline-stimulated lipogenesis and incorporation of \(^{14}\)C from [\(^{14}\)C]proline into fatty acid by 46 ± 5% and 57 ± 7% respectively. The inhibitory effects of angiotensin II on proline-stimulated lipogenesis and \(^{14}\)C-incorporation from [\(^{14}\)C]proline were observed but were rather less than those of vasopressin. Lipogenesis was inhibited by 21 ± 1% and \(^{14}\)C-incorporation from [\(^{14}\)C]proline by 27 ± 3%.
3-MPA did not significantly affect rates of lipogenesis in the presence of proline or $^{14}$C-incorporation from [U-$^{14}$C]proline into fatty acid and inhibitory effects of the hormones were observed in the presence of 3-MPA. Thus pyruvate formation from proline via phosphoenolpyruvate carboxykinase and pyruvate kinase is not quantitatively important for the incorporation of $^{14}$C from [U-$^{14}$C]proline into fatty acid.

Proline-stimulated rates of lipogenesis were inhibited by (-)HC by approximately 51% in the absence of the hormones and by $40 \pm 6\%$ (n = 5) or $43 \pm 5\%$ (n = 6) in the presence of vasopressin or angiotensin II respectively (Table 2). The inhibitory effect of vasopressin on lipogenesis was decreased in the presence of (-)HC; that of angiotensin II was not observed. (-)HC decreased incorporation of $^{14}$C from [U-$^{14}$C]proline by 90-94% both in the absence and the presence of the hormones. This almost complete inhibition of incorporation contrasts with the partial inhibition observed with cells incubated with [U-$^{14}$C]lactate and is consistent with the idea that incorporation of $^{14}$C from [U-$^{14}$C]lactate or [U-$^{14}$C]proline into fatty acid occurs via different pathways.

**Discussion**

Differences in the effects of vasopressin and angiotensin II on lipogenesis in hepatocytes from fed rats may result from net and variable glycolytic flux from glycogen or exogenous glucose. Hormonal inhibition of lipogenesis has been observed in hepatocytes prepared and incubated without glucose (Williamson et al., 1980); hormonal stimulation has been observed when glucose has been included in the perfusion and incubation media (Assimacopoulos-Jeannet et al., 1981). In the present experiments glucose was not used during cell preparation. However, we chose to incubate the cells with glucose at a concentration (5 mM) at which there is glucose-glucose 6-phosphate recycling without net glucose utilization (see Hers, 1976). Under these defined conditions, inhibition of lipogenesis by the hormones was consistently observed. In contrast to results of other groups (Clark et al., 1974; Harris, 1975; Assimacopoulos-Jeannet et al., 1981) addition of lactate and pyruvate to hepatocytes from fed rats did not increase lipogenesis measured with $^{3}$H$_2$O. Therefore, in our hands, the provision of lactate is not rate-limiting for lipogenesis. This may explain why vasopressin and angiotensin II, which increase glycolytic flux (and lactate production) secondary to increased glycogenolysis (Williamson et al., 1980) did not stimulate lipogenesis.

To differentiate between direct effects of the hormones on lipogenesis and indirect effects mediated by alterations in glycolytic flux we investigated possible effects of the hormones on lipogenesis in hepatocytes depleted of glycogen in vitro. The method used for glycogen-depletion (in vitro incubation with dibutyryl cyclic AMP) has the potential disadvantage that it may lead to acute cyclic-AMP-dependent changes in enzyme activities, which may persist after removal of the dibutyryl cyclic AMP and cause new reactions to become rate-limiting for lipogenesis. Some evidence for this occurring is that whereas (-)HC inhibits lipogenesis (and $^{14}$C incorporation into
fatty acid from [U-14C]lactate) in the presence of lactate and pyruvate in hepatocytes from fed rats, similar inhibitory effects of (-)HC are not observed in hepatocytes treated with dibutyryl cyclic AMP to deplete glycogen. Nonetheless, as with hepatocytes from fed rats, inhibition of lipogenesis by vasopressin and angiotensin II was always observed. The results with the glycogen-depleted cells thus clearly indicate, firstly, that increased glycolytic flux from glycogen is not prerequisite for the inhibitory effect of the hormones on lipogenesis, and, secondly, that pretreatment of hepatocytes with dibutyryl cyclic AMP does not prohibit the expression on this inhibition.

The inhibition of lipogenesis by angiotensin II and vasopressin observed in glycogen-depleted hepatocytes incubated with lactate provides evidence that the site of hormone action is subsequent to lactate formation. The decreased ability of the hormones to inhibit lipogenesis in the presence of (-)HC indicates that flux through ATP-citrate lyase is important in a permissive sense for the hormone effect. It is difficult to establish more precisely the major site of inhibition, because although the hormones inhibit both proline- and lactate-stimulated lipogenesis in glycogen-depleted hepatocytes (which might suggest an action at a site common to the metabolism of both these compounds) it is uncertain whether proline carbon is being used for net lipogenesis. This uncertainty arises since proline stimulates lipogenesis in the presence of (-)HC, where incorporation of 14C from [U-14C]proline into fatty acid is not observed. The finding that proline-stimulated but not lactate-stimulated lipogenesis is inhibited by (-)HC in glycogen-depleted hepatocytes indicates that lactate and proline stimulate lipogenesis by different mechanisms, and that flux through ATP-citrate lyase is only important in the former case.

Our results demonstrate that in the absence of glycolysis from glycogen, both angiotensin II and vasopressin consistently inhibit fatty acid synthesis in the absence or presence of lipogenic substrates. The effects of the hormones in vivo are likely to depend on the nutritional state of the animal, the portal glucose concentration, hepatic glycogen content, and net glycolytic flux in the liver. The hormones may also influence lipogenesis by changing hepatic blood flow (and thus availability of substrates of extrahepatic origin, and oxygen). One might speculate that acute increases in blood concentrations of angiotensin II or vasopressin in the well-fed animal, associated possibly with decreased hepatic perfusion (see Hems et al., 1976) would increase hepatic lipogenesis. During the transition from the fed to the starved state the hormones would inhibit lipogenesis. Possible changes in effects of hormones dependent on the nutritional state of the animal may warrant more consideration.

Acknowledgements

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