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Swelling of rat hepatocytes activates acetyl-CoA carboxylase in parallel to glycogen synthase

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Incubation of hepatocytes in conditions known to increase their volume, i.e. with amino acids or in hypo-osmotic media, resulted in the parallel activation of glycogen synthase and acetyl-CoA carboxylase. The activation of both enzymes by glutamine was antagonized by the addition of raffinose to prevent cell swelling, or by glucagon and microcystin. The findings are consistent with the involvement of a common mechanism for the activation of the two enzymes.

INTRODUCTION

In isolated hepatocytes, several amino acids, notably glutamine and proline, are known to stimulate glycogen synthesis [1–5]. The regulatory mechanism involved, which results in an activation of glycogen synthase, seems to be mediated by an increase in cell volume caused by the Na^+-dependent amino acid uptake [4,5]. In agreement with this interpretation, cell swelling itself has been shown to stimulate glycogen synthesis [4]. On the other hand, glutamine and proline inhibit ketogenesis and stimulate lipogenesis in isolated rat hepatocytes [3,6]. A similar, but smaller, effect on lipogenesis was also obtained when hepatocytes were incubated under hypo-osmotic conditions [6].

Lipogenesis and ketogenesis can be regulated at the level of acetyl-CoA carboxylase, which controls the concentration of malonyl-CoA, a strong inhibitor of the carnitine-dependent entry and oxidation of long-chain fatty acids in mitochondria [7]. Incubation of hepatocytes with glutamine increased malonyl-CoA concentration [6], suggesting that acetyl-CoA carboxylase was activated. This enzyme is regulated by ligands, such as citrate, and by covalent modification. Like glycogen synthase, acetyl-CoA carboxylase exists in an active dephosphorylated form and an inactive phosphorylated form; furthermore, both enzymes are regulated by multi-site phosphorylation [8–11]. On the other hand, activation of acetyl-CoA carboxylase by insulin could result from phosphorylation [12,13].

In this work, we studied whether the regulatory mechanism involved in the stimulation of lipogenesis by glutamine and proline could result from an activation of acetyl-CoA carboxylase and whether glycogen synthase and acetyl-CoA carboxylase could be activated in parallel. We also studied if hypo-osmoticity could activate acetyl-CoA carboxylase. The findings suggest a common regulatory mechanism for glycogen synthase and acetyl-CoA carboxylase activation, which could be triggered by cell swelling.

MATERIALS AND METHODS

Glucagon (Novo Nordisk), microcystin-LR (Calbiochem), radiochemicals (Amersham International), and other biochemical reagents (Sigma or Boehringer Mannheim) were purchased as indicated.

Hepatocytes were prepared as described previously [14] from overnight-fasted male Wistar rats (200–220 g). The cells were shaken (120 strokes/min) in stoppered vials at 37 °C for the times indicated. The standard incubation medium was a Krebs–Henseleit bicarbonate buffer at pH 7.4 [15]. Hypo-osmotic Na^+-depleted media were obtained by decreasing the Na^+ concentration of the buffer from 145 to 85 mM. All media were in equilibrium with a gas phase of O_2/CO_2 (19:1). The concentration of amino acids was 10 mM. The cell volume was estimated by cell weight as previously described [4].

Glycogen, lipogenesis and glycogen synthase *a* were measured as previously reported [3]. The method generally used to measure acetyl-CoA carboxylase activity in crude cell extracts is subject to criticism. Indeed, it relies on the measurement of the incorporation of radioactive CO_2 into acid-soluble compounds, which is non-specific, since the activities of other carboxylases, such as pyruvate carboxylase, are also measured [16]. To circumvent this difficulty, we have used an assay described elsewhere [17], which is based on the measurement of the incorporation of radioactive acetyl units into lipids by coupling acetyl-CoA carboxylase activity to an excess of fatty acid synthase added to permeabilized hepatocytes. The assay is performed as recommended [17] with a physiological concentration [3,18] of citrate (0.5 mM), a known stimulator of acetyl-CoA carboxylase [9]. Phosphorylation by cyclic AMP-dependent protein kinase or AMP-activated protein kinase inactivates acetyl-CoA carboxylase by decreasing $V_{max.}$ and increasing the K_a for citrate from about 2 mM to 4 or 10 mM, depending on the kinase [19,20]; thus the citrate concentration used in the assay is below the K_a reported for the phosphorylated and dephosphorylated forms [19,20]. Rat liver fatty acid synthase was purified as described previously [21].

The results are expressed as means ± S.E.M. for observations on the indicated number (*n*) of different cell preparations. Statistical significance of differences was calculated by Student's *t* test for paired data.

RESULTS AND DISCUSSION

Incubation of isolated hepatocytes with 10 mM-amino acids, such as glutamine, proline or alanine, activated glycogen synthase, as expected [2–4]. it also activated acetyl-CoA carboxylase (Table 1). Similarly, incubation of hepatocytes under hypo-osmotic conditions, in a Na^+-depleted medium, induced an activation of both glycogen synthase and acetyl-CoA carboxylase (Table 1), suggesting that the activation mechanism is a common process set off by swelling.

In these cells, an activation of glycogen synthase corresponded to the stimulation of glycogen synthesis, whereas the stimulation of lipogenesis correlated with the activation of acetyl-CoA carboxylase only when cells were incubated with proline, glutamine or alanine. In cells incubated in hypo-osmotic media, the stimulation of lipogenesis was smaller than expected from the activation of acetyl-CoA carboxylase (Table 1). This confirms

Table 1. Synthase *a*, acetyl-CoA carboxylase, glycogen synthesis and lipogenesis in hepatocytes incubated with various amino acids or in hypo-osmotic medium

Hepatocytes were incubated with 20 mM-glucose and 10 mM-amino acids. Synthase *a* and acetyl-CoA carboxylase were measured in samples taken after 30 min of incubation. The rates of glycogen and lipid synthesis correspond to the glycogen or lipids formed between 0 and 60 min. The values are means ± S.E.M. for (*n*) cell preparations: *significantly different ($P < 0.05$) from the control values.

	Synthase *a* (m-units/g of cells)	Acetyl-CoA carboxylase (m-units/g of cells)	Glycogen synthesis (μmol of glucose/h per g of cells)	Lipogenesis (μmol of ^3H_2O/h per g of cells)
Control	189 ± 13 (13)	3.36 ± 0.45 (16)	1.83 ± 0.35 (4)	0.56 ± 0.06 (6)
Glutamine	309 ± 24* (13)	9.30 ± 1.56* (16)	11.36 ± 3.10* (4)	2.64 ± 0.42* (6)
Proline	494 ± 77* (6)	14.19 ± 3.57* (10)	11.10 ± 2.57* (4)	2.76 ± 0.48* (6)
Alanine	348 ± 49* (4)	7.57 ± 2.10* (6)	7.17 ± 1.73* (4)	1.51 ± 0.41* (6)
Leucine	228 ± 43 (4)	11.54 ± 2.25* (5)	2.29 ± 0.63 (4)	1.09 ± 0.24* (6)
Na^+-depleted medium	348 ± 26* (4)	10.87 ± 1.74* (6)	6.05 ± 1.14* (4)	0.88 ± 0.09* (6)

Table 2. Additivity of the effects of glutamine and of hypo-osmotic medium on cell swelling, synthase *a* and acetyl-CoA carboxylase in hepatocytes

Hepatocytes were incubated with 20 mM-glucose and 10 mM-glutamine. Cell weight and enzyme activities were measured in samples taken after 30 min of incubation. The values are means ± S.E.M. for four cell preparations: *significantly different ($P < 0.05$) from the control values; †significantly different ($P < 0.05$) from the values in the presence of glutamine or in the Na^+-depleted medium.

	Cell weight (% of control)	Synthase *a* (m-units/g of cells)	Acetyl-CoA carboxylase (m-units/g of cells)
Control	100	233 ± 18	2.72 ± 0.22
Glutamine	118.2 ± 1.7*	363 ± 17*	6.26 ± 1.16*
Na^+-depleted medium	118.3 ± 3.7*	378 ± 13*	6.43 ± 0.79*
Na^+-depleted medium + glutamine	141.8 ± 6.4†	468 ± 21†	13.84 ± 2.88†

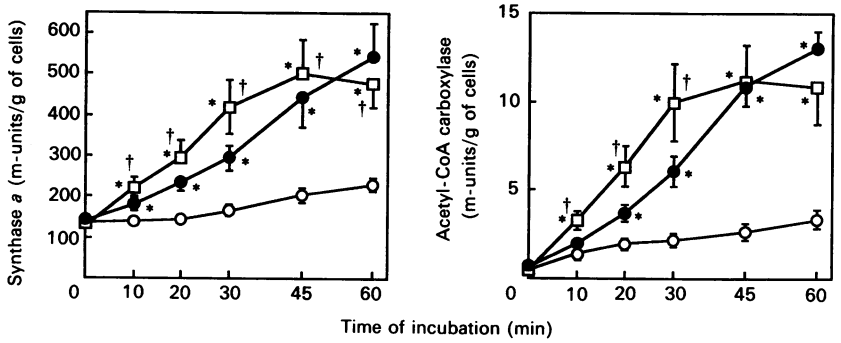

Fig. 1. Time course of activation of glycogen synthase and acetyl-CoA carboxylase by glutamine or proline in hepatocytes

Hepatocytes were incubated with 20 mM-glucose without further addition (○) or with 10 mM-glutamine (●) or proline (□) for the indicated periods of time. The values are means ± S.E.M. for seven cell preparations: *significantly different ($P < 0.05$) from the corresponding control values; †significantly different ($P < 0.05$) from the corresponding values in the presence of glutamine.

our previous observation that the stimulation of lipogenesis by hypo-osmoticity was less than with glutamine or proline, although swelling and stimulation of glycogen synthesis were similar under these conditions [6]. Moreover, our previous results [6] showed that the effects of glutamine and of hypo-osmotic medium on both volume changes and glycogen synthesis were additive, whereas those on lipogenesis were not: incubation in Na^+-depleted medium could even decrease the stimulation of lipogenesis by glutamine. We now show in Table 2 that the effects of hypo-osmoticity and glutamine on synthase and acetyl-CoA carboxylase activation are additive. The contradiction between the effects of swelling induced by glutamine and Na^+-depleted medium is only apparent, and disappears if one considers the effects of swelling on the activation of both enzymes. Therefore, in hepatocytes incubated in hypo-osmotic medium, regulatory factors other than acetyl-CoA carboxylase are actually controlling the lipogenic flux.

Incubation of hepatocytes with leucine, which does not increase cell volume [4], failed to activate glycogen synthase as expected [4], whereas it was able to activate acetyl-CoA carboxylase and

Table 3. Inhibition of cell swelling, and of synthase and acetyl-CoA carboxylase activation, by hyperosmotic medium in hepatocytes

Hepatocytes were incubated with 20 mM-glucose, 10 mM-glutamine and 80 mM-raffinose as indicated. Cell weight and enzyme activities were measured in samples taken after 30 min incubation. The values are means ± S.E.M. for four (cell weight) or six (others) cell preparations: *significantly different ($P < 0.05$) from the control values; †significantly different ($P < 0.05$) from values in the presence of raffinose alone.

	Cell weight (% of control)	Synthase *a* (m-units/g of cells)	Acetyl-CoA carboxylase (m-units/g of cells)
Control	100	160 ± 15	3.26 ± 0.76
Raffinose	98.4 ± 1.7	129 ± 15	2.50 ± 0.90
Glutamine	112.2 ± 2.1*	263 ± 23*	10.51 ± 1.97*
Glutamine + raffinose	101.7 ± 2.7†	179 ± 21†	2.81 ± 0.90

Table 4. Inhibition by glucagon or microcystin of synthase and acetyl-CoA carboxylase activation by glutamine without decreasing cell volume

Hepatocytes were incubated with 20 mM-glucose, 10 mM-glutamine and 100 nM-glucagon or 100 nM-microcystin as indicated. Enzyme activities and cell weight were measured in samples taken after 45 min incubation (microcystin), or 5 min after addition of glucagon. The hormone was added after 30 min incubation. The values are means ± S.E.M. for three (cell weight) or four (others) cell preparations: *significantly different ($P < 0.05$) from values in absence of added substances; †significantly different ($P < 0.05$) from values in absence of glutamine but in presence of glucagon or microcystin.

	Cell weight (% of value in absence of added substance)		Synthase *a* (m-units/g of cells)		Acetyl-CoA carboxylase (m-units/g of cells)	
	Control	Glutamine	Control	Glutamine	Control	Glutamine
Control	100	109.4 ± 0.6*	166 ± 16	299 ± 39*	2.91 ± 0.69	7.33 ± 2.20*
Glucagon	99.3 ± 0.3	109.2 ± 1.2†	75 ± 13*	124 ± 24†	0.51 ± 0.25*	0.86 ± 0.27
Control	100	113.5 ± 1.3*	221 ± 20	450 ± 34*	3.55 ± 0.64	14.31 ± 2.37*
Microcystin	103.5 ± 0.3*	128.5 ± 3.6†	101 ± 7*	150 ± 6†	1.55 ± 0.62*	3.57 ± 0.51†

to stimulate lipogenesis slightly (Table 1). These data indicate that the activation of acetyl-CoA carboxylase by leucine is clearly not mediated by swelling.

The time courses of activation of glycogen synthase and acetyl-CoA carboxylase in hepatocytes incubated with either glutamine or proline were compared. Fig. 1 shows a time-dependent and parallel activation of both synthase and acetyl-CoA carboxylase after the addition of each amino acid. The data confirm the faster effect of proline on synthase activation and on glutamate and aspartate accumulation [6]. In addition, they show that this difference in the rate of activation of synthase by proline was also observed for the activation of acetyl-CoA carboxylase. The parallelism between the activation of both enzymes is striking.

We previously reported that the activation of synthase by glutamine could be mediated by cell swelling. Addition of 80 mM-raffinose to the incubation medium was indeed able to antagonize the activation of synthase by glutamine [4]. We have repeated this type of experiment, and Table 3 shows that the activation of both synthase and acetyl-CoA carboxylase by glutamine could be antagonized by the addition of raffinose to the incubation medium. In the same cells, the change in cell volume confirmed that the addition of raffinose could prevent the 12% increase in cell volume induced by glutamine, and resulted in a volume which was similar to that of control cells (Table 3).

The activation of acetyl-CoA carboxylase could result from covalent modification or from interaction with ligands, such as citrate, a known stimulator of acetyl-CoA carboxylase [9]. Since the concentration of citrate is decreased in hepatocytes treated with glutamine [3], it is not possible to link an increase in acetyl-CoA carboxylase activity to the observed change in citrate concentration. Therefore, we tested the possibility that the activation of both synthase and acetyl-CoA carboxylase by glutamine could result from covalent modification. If so, the activation should be antagonized by either glucagon or microcystin. Glucagon acts via the cyclic AMP-dependent protein kinase to inactivate both glycogen synthase and acetyl-CoA carboxylase by phosphorylation [10,11]. Whether the mechanism of inactivation is direct or indirect, as recently suggested [11], does not really matter in this context. On the other hand, microcystin is a known inhibitor of the protein phosphatases that are able to activate glycogen synthase and acetyl-CoA carboxylase [22,23]. The results, presented in Table 4, show that both glucagon and microcystin diminished the activation of synthase and acetyl-CoA carboxylase without decreasing the volume of the cells. On the contrary, microcystin increased the volume of glutamine-treated cells (Table 4) This is in agreement with the observed inhibition of swelling-activated KCl efflux by okadaic acid [24,25], which is another known inhibitor of protein phosphatase [26]. Therefore, we suggest that the glutamine-induced activation of acetyl-CoA carboxylase, like that of synthase, results from a covalent modification, namely a dephosphorylation of the enzyme, which was antagonized by glucagon and microcystin.

Conclusion

Glutamine and proline stimulate glycogen synthesis and lipogenesis by activation of glycogen synthase and acetyl-CoA carboxylase respectively. The striking similarity between their effects suggests the involvement of a common regulatory mechanism, which could be triggered by cell swelling. This interpret-

ation is based on the inhibition of both glycogen synthase and acetyl-CoA carboxylase activation by hyperosmotic medium, and on the fact that swelling in hypo-osmotic medium (i.e. in the absence of amino acids) also activates both enzymes. Thus cell swelling can be regarded as a signal controlling the activation of glycogen synthase and acetyl-CoA carboxylase. Other studies have shown that cell swelling has other metabolic effects, such as inhibition of proteolysis and activation of amino acid transport by the N transporter [27–32].

The precise nature of the molecular mechanism linking cell swelling to glycogen synthase and acetyl-CoA carboxylase activation is not known. It could result from the stimulation of a protein phosphatase common to the activation of both enzymes. According to Cohen [22], protein phosphatase 2A, which is inhibited by microcystin, is supposed to be responsible for acetyl-CoA carboxylase activation. The same phosphatase can also activate glycogen synthase, although the phosphatase mainly involved in synthase activation is a different enzyme, called protein phosphatase 1G [22,33]. Alternatively, the mechanism could be a common regulator for these different phosphatases acting respectively on synthase and acetyl-CoA carboxylase. Finally, we cannot exclude the possibility that control could be exerted at the level of AMP-activated protein kinase, which is known to inactivate acetyl-CoA carboxylase and which has been proposed to be involved in glucagon-mediated inactivation of acetyl-CoA carboxylase in hepatocytes [8,11].

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