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Stimulation of glycogen synthesis and lipogenesis by glutamine in isolated rat hepatocytes

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Glutamine stimulated glycogen synthesis and lactate production in hepatocytes from overnight-fasted normal and diabetic rats. The effect, which was half-maximal with about 3 mM-glutamine, depended on glucose concentration and was maximal below 10 mM-glucose. β-2-Aminobicyclo[2.2.1]heptane-2-carboxylic acid, an analogue of leucine, stimulated glutaminase flux, but inhibited the stimulation of glycogen synthesis by glutamine. Various purine analogues and inhibitors of purine synthesis were found to inhibit glycogen synthesis from glucose, but they did not abolish the stimulatory effect of glutamine on glycogen synthesis. The correlation between the rate of glycogen synthesis and synthase activity suggested that the stimulation of glycogen synthesis by glutamine depended solely on the activation of glycogen synthase. This activation of synthase was not due to a change in total synthase, nor was it caused by a faster inactivation of glycogen phosphorylase, as was the case after glucose. It could, however, result from a stimulation of synthase phosphatase, since, after the addition of 1 nM-glucagon or 10 nM-vasopressin, glutamine did not interfere with the inactivation of synthase, but did promote its subsequent re-activation. Glutamine was also found to inhibit ketone-body production and to stimulate lipogenesis.

INTRODUCTION

In isolated liver preparations, glucose is a poor substrate for glycogen synthesis when given at concentrations below 10 mM [1–3]. However, efficient glycogen synthesis can readily be achieved if gluconeogenic precursors such as fructose, dihydroxyacetone, glycerol or lactate are added to the preparations [4–8]. These precursors not only provide carbon atoms for glycogen synthesis, but also promote the activation of glycogen synthase. Glycogen synthesis from these gluconeogenic precursors is further enhanced when hepatocytes are incubated in the presence of amino acids such as glutamine, asparagine or alanine. Glutamine, which does not seem to serve as carbon source for glycogen, also causes an activation of glycogen synthase, but the exact mechanism of its action is still obscure [9–16].

Our project was to re-investigate the effect of glutamine. We have chosen to concentrate on glutamine because, in earlier work, as well as in our preliminary experiments, glutamine was found to be a better stimulator of glycogen synthesis than alanine or asparagine. For simplicity we studied the effect of glutamine in the presence of glucose alone but in the absence of other gluconeogenic precursors. Several problems have been examined. (i) What is the effect of glutamine on metabolic fluxes leading to, and coming from, glucose 6-phosphate? Does a stimulation of glucokinase and/or an inhibition of glucose-6-phosphatase flux participate in the overall stimulation of glycogen synthesis? (ii) Could an intermediate of purine metabolism, for which glutamine is an essential precursor, mediate the glutamine effect? (iii) What is the mechanism of activation of glycogen synthase by glutamine? Is it similar to that of glucose, namely activation of glycogen synthase secondary to inactivation of glycogen phosphorylase [17,18]? (iv) Is the effect of glutamine limited to the stimulation of glycogen synthesis, or is it more general in promoting other anabolic processes?

MATERIALS AND METHODS

Biochemicals

Azaserine, glutamine, 6-mercaptopurine, 6-methylmercaptopurine riboside, vasopressin (all from Sigma), methotrexate (Janssen Chimica), β-2-aminobicyclo-[2.2.1]heptane-2-carboxylic acid (BCH; Calbiochem), radiochemicals (Amersham), glucagon (Novo-Industri A/S, Copenhagen, Denmark), and other biochemical reagents (Sigma or Boehringer) were purchased as indicated.

Preparation and incubation of hepatocytes

Hepatocytes were prepared as described previously [19] from overnight-fasted male Wistar rats which were either normal or diabetic [streptozotocin (55 mg/kg) injected intravenously 3–4 weeks before the experiments]. The cells (usually 100 mg wet wt. in 2 ml) were shaken (120 strokes/min) in stoppered 20 ml flasks at 37 °C for the times indicated. The gas phase was O_2/CO_2 (19:1). For the determination of glycogen, samples (0.5 ml) of the cell suspension were pipetted into tubes containing 10 ml of ice-cold 0.15 M-NaCl, centrifuged (30 s at 2000 *g*, table centrifuge), and the pellet was resuspended in 0.5 ml of 0.5 M-NaOH. The tubes were heated for

Abbreviation used; BCH, β-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid.

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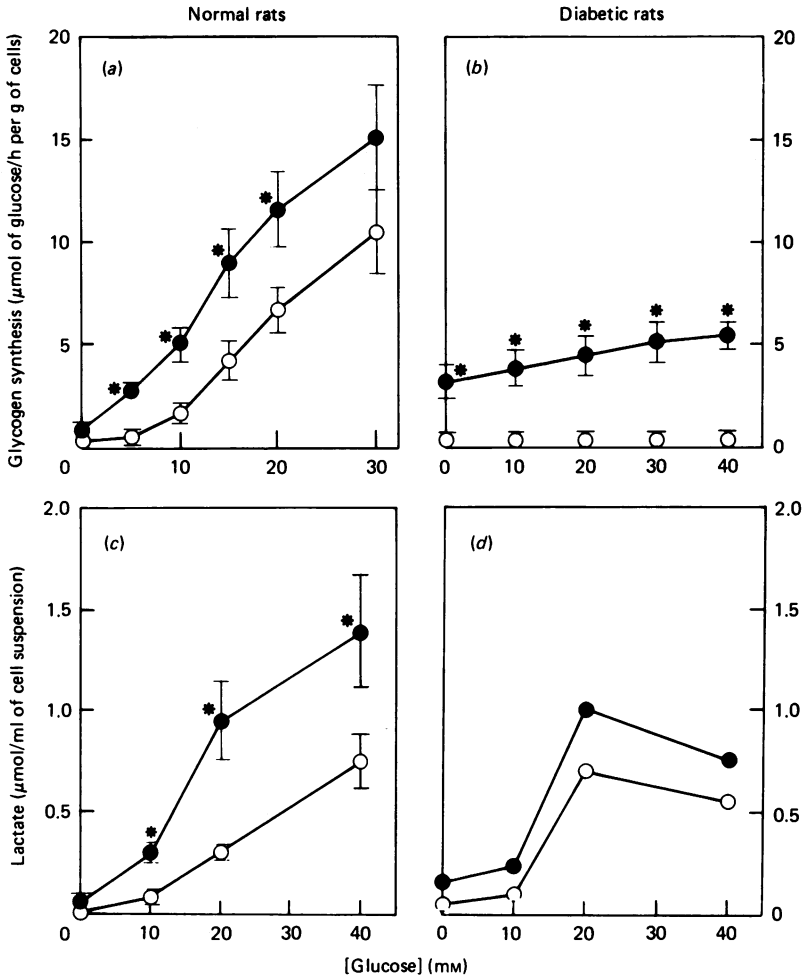

Fig. 1. Stimulation of glycogen synthesis and lactate production by 10 mM-glutamine in hepatocytes from normal and diabetic rats

After incubation for 20 min with the indicated concentrations of glucose, 10 mM-glutamine was added and the incubation was continued for 1 h to measure glycogen synthesis (*a* and *b*). For the measurement of lactate (*c* and *d*), the cells were incubated for 30 min with 10 mM-glutamine and glucose as indicated. The values are means ± S.E.M. for three or four cell preparations, except in (*d*), where the means for two cell preparations are shown. ○, Controls; ●, +glutamine. *Significantly different ($P < 0.05$) from values in the absence of glutamine.

15 min at 100 °C and, after cooling, 0.2 ml of 2 M-acetic acid was added; the precipitated proteins were eliminated by centrifugation (table centrifuge) and the pH of the supernatant was adjusted to about 6 by addition of 0.04 ml of 2 M-NaOH. Samples were incubated for 30 min at 37 °C with amyloglucosidase (0.1 mg/ml) in 0.1 M-acetate, pH 5 [2], and the glucose produced was measured by the glucose oxidase method [20]. For the measurement of metabolites, the incubation was stopped by the addition of 0.5 ml of 2 M-$HClO_4$. For the measurement of ^3H_2O release from [2-^{3}H]- and [3-^{3}H]-glucose, ^3H_2O was separated from the radioactive glucose in $HClO_4$ extracts [21]. The isolation of radioactive glucose formed from [U-^{14}C]fructose or [U-^{14}C]galactose was performed as described previously [19]. For the measurement of lipogenesis, ^3H_2O (0.1 mCi/ml) was added after 20 min of incubation, and the incorporation of radioactivity into fatty acids was measured [22] in samples taken 50 min after this addition. To measure enzyme activities, samples (0.1 ml) were taken at the times indicated, mixed with 0.02 ml of 0.6 M-KF/0.12 M-EDTA/3% (w/v) glycogen/0.05 M-glycylglycine, pH 7.4, and immediately frozen in a cooling mixture (solid CO_2 in acetone).

Assays

Glucose [20], glucose 6-phosphate [23], β-hydroxybutyrate [24], acetoacetate [25], lactate [26] and citrate [27] were measured enzymically in neutralized $HClO_4$ extracts as indicated in the references. Glycogen synthase activity was measured [28] at 25 °C in the presence of 5 mM-UDP-[U-^{14}C]glucose, 1% (w/v) glycogen, 4 mM-EDTA, 20 mM-KF, 50 mM-Hepes, pH 7.8, and 10 mM-Na_2SO_4 (synthase *a*) or 10 mM-glucose 6-phosphate (synthase *a*+*b*). Phosphorylase activity was measured as described [2], but at 25 °C. Enzyme activity is expressed in units, i.e. μmol of substrate transformed/min under the assay conditions.

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

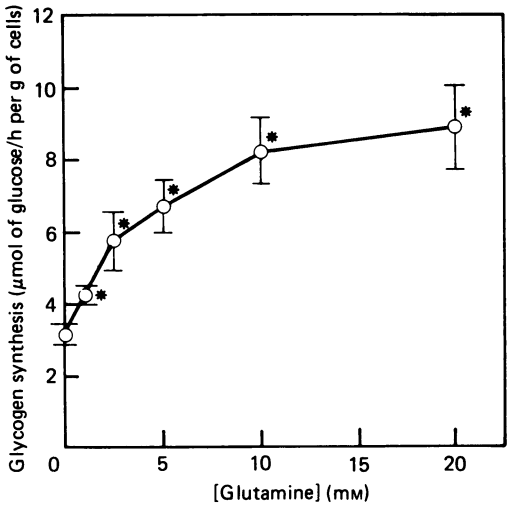

Fig. 2. Dose/response of the effect of glutamine on the synthesis of glycogen in hepatocytes from normal rats

Same protocol as in Fig. 1(*a*). The values are means ± S.E.M. for three cell preparations. *Significantly different ($P < 0.02$) from values in the absence of glutamine.

Table 1. Influence of 10 mM-glutamine on detritiation of [2-^{3}H]- and [3-^{3}H]glucose, and on conversion of [U-^{14}C]fructose and [U-^{14}C]galactose into glucose, in hepatocytes from normal rats

For the measurement of glucose detritiation, the cells were incubated for 20 min with 20 mM-glucose, then 10 mM-glutamine and the radioactive glucose tracer (0.5 μCi/ml) were added, and the incubation was continued for 30 min. For the measurement of glucose formation from fructose and galactose, the cells were incubated for 20 min with 20 mM-glucose; 10 mM-glutamine was then added and, 20 min later, the radioactive precursors (0.25 μCi/ml; 0.5 mM final concn.) were added and the incubation was continued for 10 min. *Significantly different ($P < 0.05$) from values in the absence of glutamine.

	Rate (nmol of glucose/min per g of cells)	
	Control	+Glutamine
Detritiation of [2-^{3}H]glucose	505 ± 50 (4)	510 ± 3 (4)
Detritiation of [3-^{3}H]glucose	240 ± 13 (4)	270 ± 10 (4)
Conversion of [U-^{14}C]fructose into glucose	117 ± 11 (3)	81 ± 4* (3)
Conversion of [U-^{14}C]galactose into glucose	133 ± 4 (3)	132 ± 4 (3)

RESULTS AND DISCUSSION

Effects of glutamine on carbohydrate metabolism

The stimulation of glycogen synthesis by glutamine was confirmed. In hepatocytes from normal rats, the extent of stimulation of glycogen synthesis and lactate production by 10 mM-glutamine (Figs. 1*a* and 1*c*) depended on glucose concentration. The largest effect was obtained within the physiological range, i.e. below 10 mM-glucose. In the presence of 20 mM-glucose, half-maximal stimulation of glycogen synthesis was obtained with about 3 mM-glutamine (Fig. 2). In hepatocytes from diabetic rats, net glycogen synthesis was not observed with glucose alone, as expected [6,29,30]. It was, however, obtained with 10 mM-glutamine, and even in the absence of glucose (Fig. 1*b*). In the latter situation, the effect of glutamine was much greater in diabetics than in control rats, and glucose was not required to obtain a stimulation of glycogen synthesis by glutamine.

Measurement of the release of ^3H_2O from [2-^{3}H]- and [3-^{3}H]-glucose, which can be used to estimate glucose flux through glucokinase and phosphofructokinase respectively [31], showed that glutamine did not increase glucose flux in hepatocytes from normal rats (Table 1). Therefore, the additional lactate that was released in the presence of glutamine when the glucose concentration was increased (Figs. 1*c* and 1*d*), probably originated from glutamine rather than from glucose. Glucose is indeed known to inhibit gluconeogenesis through the inhibitory effect of fructose 2,6-bisphosphate on fructose-1,6-bisphosphatase [32]. Consistent with this interpretation is the fact that the concentration of fructose 2,6-bisphosphate in hepatocytes incubated with 20 mM-glucose was not affected by 1–10 mM-glutamine (results not shown). On the other hand, the formation of glucose from radioactive fructose was compared with that from galactose, in the presence and the absence of glutamine. Glucose-6-phosphatase, which catalyses the only common step in the metabolism of these two glucose precursors, is usually regarded as 'rate-limiting' for the formation of glucose, and any inhibition of glucose-6-phosphatase should affect glucose formation from the two precursors. As shown in Table 1, glutamine inhibited (35%) the formation of glucose from 0.5 mM-fructose, but had no effect on the conversion of 0.5 mM-galactose into glucose. Although the evidence is indirect, comparison of these results suggests that glutamine does not inhibit the flux through glucose-6-phosphatase. In addition, the fact that glutamine did not change glucose 6-phosphate concentration (Table 2) reinforces this hypothesis. This conclusion is also supported by the relationship between synthase activity and glycogen synthesis shown in Fig. 3. If glutamine had not only activated synthase but also influenced other steps in the pathway, one would have expected that, for the same synthase activity, the amount of glycogen synthesized would be larger in the presence of glutamine. Since this was not the case, the stimulation of glycogen synthesis by glutamine can be attributed solely to the activation of synthase.

BCH, an analogue of leucine, has been shown to stimulate glucose and urea formation from glutamine in hepatocytes [33]. This has been explained by an activation of the mitochondrial glutaminase [34]. The results shown in Fig. 4 confirm that 10 mM-BCH [similar results (not shown) were obtained with 5 mM-BCH] indeed stimulated glucose formation from glutamine, but they also demonstrate that BCH inhibited the stimulatory effect of glutamine on glycogen synthesis, indicating that the two processes are not necessarily linked. These results also suggest that the intermediate responsible for the stimulation of glycogen synthesis by glutamine could be formed in a reaction before the step catalysed by glutaminase. Since purine synthesis is one of the possible

Table 2. Influence of glutamine on the concentration of glucose 6-phosphate and citrate in isolated hepatocytes from normal rats

The cells were incubated for 30 and 60 min with or without 10 mM-glutamine and in the presence of glucose at the indicated concentrations. *Significantly different ($P < 0.05$) from values in the absence of glutamine.

[Glucose] (mM)	Glutamine	Metabolite concn. (nmol/g of cells)			
		Glucose 6-phosphate		Citrate	
		30 min	60 min	30 min	60 min
10	−	67±7 (4)	63±6 (8)	−	224±51 (6)
10	+	65±6 (4)	72±7 (8)	−	266±40 (6)
20	−	83±9 (6)	88±7 (8)	500±92 (5)	374±32 (9)
20	+	84±9 (6)	95±8 (8)	277±67* (5)	282±38* (9)
40	−	114±11 (4)	135±11 (7)	−	365±39 (6)
40	+	112±10 (4)	139±13 (7)	−	280±67 (6)

pathways for glutamine utilization, the interaction between glycogen synthesis and purine synthesis was studied by using several inhibitors of purine synthesis and purine analogues. These substances, used at concentrations known to inhibit purine synthesis [35,36], decreased glycogen synthesis both in control conditions (i.e. with glucose) and in the presence of glutamine (Table 3). However, at low concentrations of these substances, the effect of glutamine on glycogen synthesis persisted, suggesting that there was no relationship between the two pathways. Incubation of isolated hepatocytes with these inhibitors caused an activation of phosphorylase that offers an explanation for the inhibition of glycogen synthesis (results not shown).

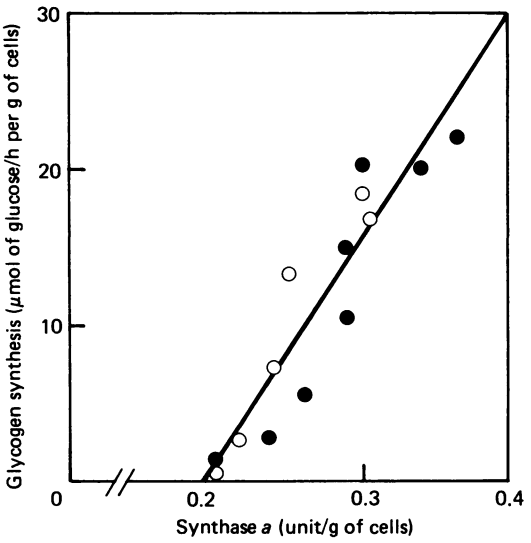

Fig. 3. Correlation between the rate of glycogen synthesis and the activity of synthase *a* in normal rat hepatocytes incubated with 10 mM-glutamine and various concentrations of glucose

Same protocol as in Fig. 1(*a*). Synthase activity was measured in samples taken after 50 min of incubation. ○, Controls; ●, +glutamine. Correlation coefficient = 0.93.

Mechanism of activation of glycogen synthase by glutamine

A series of experiments was carried out to explore the mechanism responsible for activation of glycogen synthase by glutamine. The proportion of synthase in the active (*a*) form depends on the balance between the activities of the respective kinases and phosphatases. Activation of synthase by glutamine could result from a faster inactivation of glycogen phosphorylase, as is the case after glucose administration. Phosphorylase *a* indeed inhibits synthase phosphatase, and synthase activation is observed only when the residual phosphorylase *a* activity is below a certain 'threshold' value which corresponds to about 15% of total phosphorylase [17,18]. Fig. 5(*a*) shows that 10 mM-glutamine shifted the glucose response curve of synthase activation towards lower glucose concentrations. This is similar to the profile for the effect of glutamine on glycogen synthesis (Fig. 1). It is noteworthy that total synthase activity was not increased by glutamine treatment [0.60±0.03 unit/g in controls ($n = 6$), 0.64±0.03 unit/g in glutamine-treated cells ($n = 6$)]. This is at variance with previous reports [11,14], and could result from differences in assay conditions [37]. Comparison of the time course of phosphorylase inactivation and synthase activation indicates that, in the presence of 20 mM-glucose, 10 mM-glutamine did not stimulate phosphorylase inactivation (Fig. 5*e*), but was able to cause a larger activation of synthase than did glucose alone (Fig. 5*c*). Similar results (not shown) were obtained at other glucose concentrations. In addition, in hepatocytes from diabetic rats, glucose is unable to activate synthase, in spite of the almost complete inactivation of phosphorylase, and activation of synthase was easily demonstrated in the presence of glutamine (Fig. 5*d*). In these cells, the glucose-dependent inactivation of phosphorylase was unaffected by glutamine (Fig. 5*f*). The conclusion from

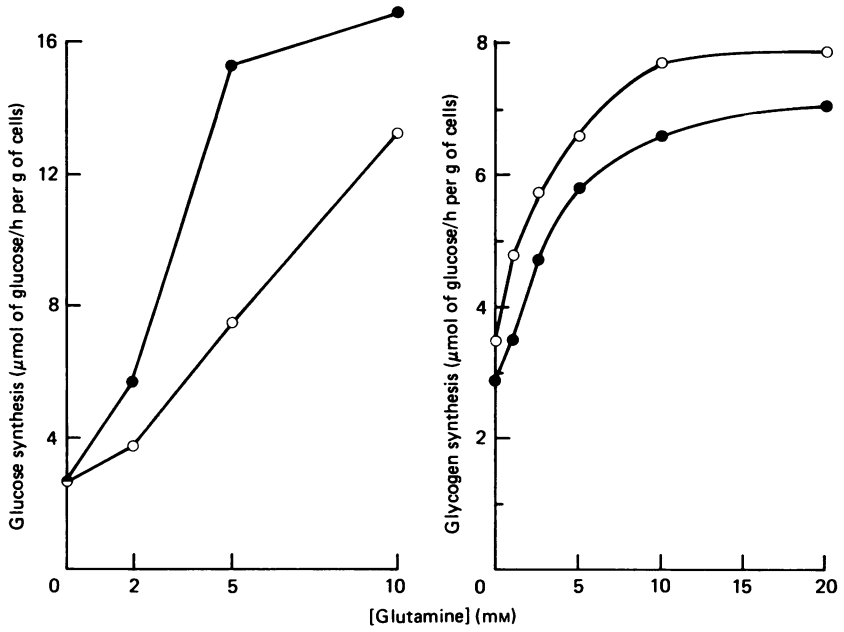

Fig. 4. Interaction of BCH with glucose formation and stimulation of glycogen synthesis by glutamine in hepatocytes from normal rats

(*a*) After incubation for 20 min, 10 mM-BCH was added with the indicated concentrations of glutamine and the incubation was continued for 1 h. (*b*) After incubation for 20 min with 20 mM-glucose, 10 mM-BCH was added together with the indicated concentrations of glutamine, and the incubation was continued for 1 h. The values are means for two cell preparations. ○, Controls; ●, +BCH.

Table 3. Effect of various inhibitors of purine synthesis and purine derivatives on the influence of glutamine on glycogen synthesis in hepatocytes from normal rats

After incubation for 20 min with 20 mM-glucose, the indicated compounds were added and the incubation was continued for 1 h.

		Glycogen synthesis (μmol of glucose/ h per g of cells)			
Addition	Concn. (mM)	Without glutamine		With 10 mM-glutamine	
Azaserine	0	6.7±0.9	(4)	15.1±1.6	(4)
	0.1	4.7	(1)	10.8	(1)
	1	3.6±0.6	(4)	9.5±1.7	(4)
Methotrexate	0	7.2±1.6	(3)	15.7±2.3	(3)
	0.1	1.0	(2)	5.9	(2)
	1	0.2	(1)	0.1	(1)
6-Mercaptopurine	0	1.1	(2)	4.4	(2)
	0.1	0.5	(2)	2.1	(2)
	1	0.1	(2)	1.2	(2)
6-Methylmercaptopurine riboside	0	6.2±1.7	(3)	13.7±2.3	(3)
	0.005	0.1	(1)	2.5	(1)
	0.01	0	(1)	0.6	(1)
	0.1	0	(2)	0	(2)
Adenosine	0	8.1±0.9	(3)	15.8±2.3	(3)
	0.05	5.8	(1)	10.9	(1)
	0.5	0.4±0.1	(3)	5.6±1.1	(3)
Inosine	0	7.0	(2)	13.0	(2)
	0.1	6.7	(2)	13.3	(2)
	0.5	6.7	(2)	12.1	(2)
	1	3.5	(1)	9.1	(1)

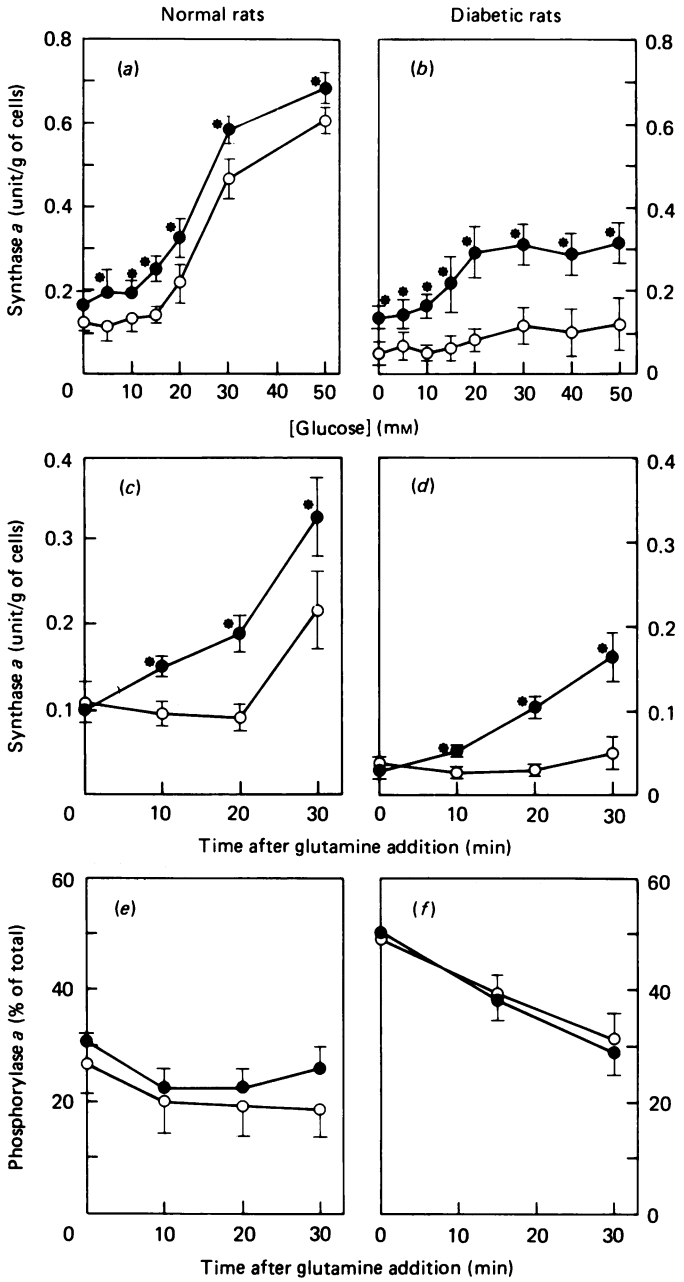

Fig. 5. Effect of 10 mM-glutamine on synthase and phosphorylase activity in hepatocytes from normal and diabetic rats

After incubation for 20 min with the indicated concentrations of glucose, 10 mM-glutamine was added and the incubation was continued for 45 min (*a* and *b*) or for the indicated periods of time (*c–f*). The concentration of glucose was 20 mM (*c*, *e*) or 10 mM (*d*,*f*). The values are means ± S.E.M. for at least four cell preparations. Total phosphorylase activity was 14.1 (normal) and 11.7 (diabetic) units/g of cells. ○, Controls; ●, +glutamine. *Significantly different ($P < 0.05$) in the presence of glutamine.

these experiments is that a 'glucose-like' mechanism cannot be invoked to explain the activation of synthase by glutamine.

A stimulation of synthase phosphatase and/or an inhibition of synthase kinase by glutamine or its metabolites remain as other plausible mechanisms. Therefore the effect of glutamine treatment on synthase inactivation was tested. Hepatocytes were incubated with or without 10 mM-glutamine and in the presence of 50 mM-glucose to activate glycogen synthase. Then, they were treated with an almost saturating concentration of glucagon (1 nM) or vasopressin (10 nM), and synthase inactivation was measured. The initial rate of synthase inactivation was not modified by glutamine (Fig. 6), indicating that the inactivation process, and hence the kinases involved [38], were not inhibited by glutamine. However, the rate of re-activation of synthase was greater (Fig. 6), suggesting that, in the presence of glutamine, kinase activity was antagonized by a more active synthase phosphatase. Thus it seems that glutamine stimulates synthase phosphatase rather than inhibiting synthase inactivition.

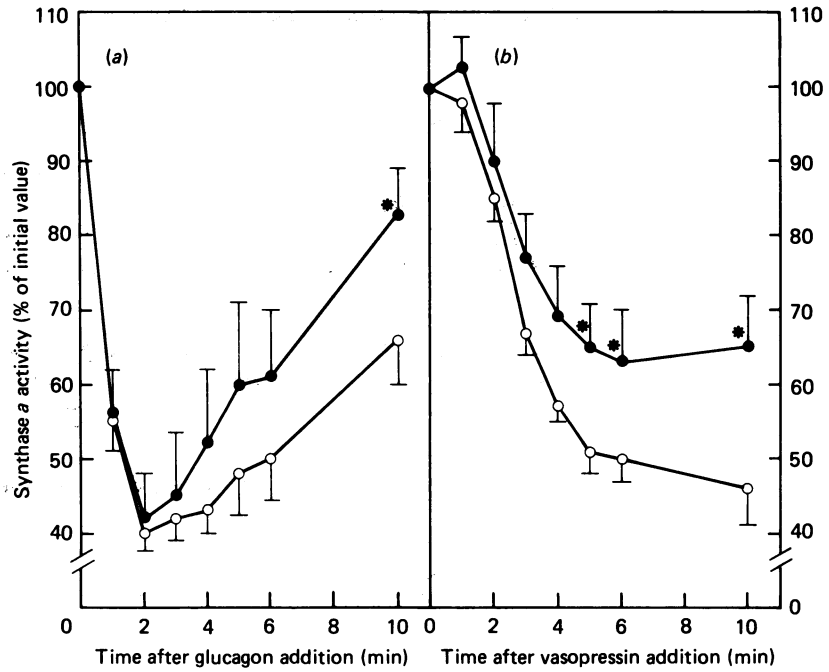

Fig. 6. Influence of 10 mM-glutamine on the effect of 1 nM-glucagon and 10 nM-vasopressin on the activity of synthase *a* in hepatocytes from normal rats

The cells were incubated for 30 min in the presence of 60 mM-glucose (○) or 50 mM-glucose and 10 mM-glutamine (●) before the addition of the hormones. The results are expressed as percentages of synthase *a* activity at zero time; this was 0.74 (*a*) and 0.80 (*b*) unit/g of cells. The values shown are means ± S.E.M. for four (*a*) and six (*b*) cell preparations. *Significantly different ($P < 0.05$) from values in the absence of glutamine.

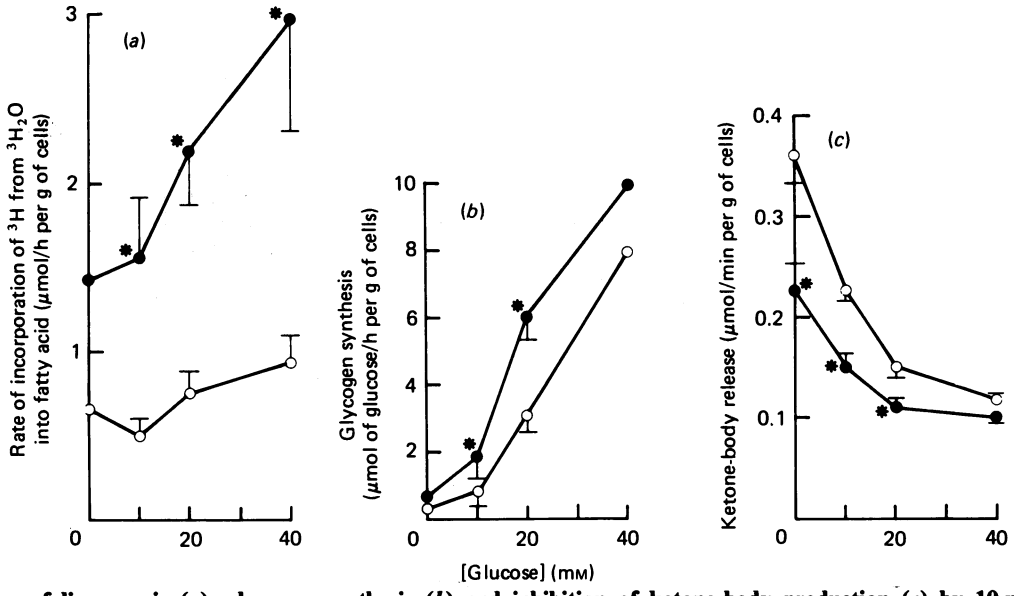

Fig. 7. Stimulation of lipogenesis (*a*), glycogen synthesis (*b*) and inhibition of ketone-body production (*c*) by 10 mM-glutamine in hepatocytes from normal rats

Same protocol as in Fig. 1(*a*). The values are means ± S.E.M. for five cell preparations. *Significantly different ($P < 0.05$) from values in the absence of glutamine.

Effect of glutamine on lipid metabolism

The influence of glutamine on ketone-body production was studied in hepatocytes incubated with various glucose concentrations. Glutamine reinforced the glucose-dependent decrease in ketone-body production (Fig. 7*c*). The effect was maximal at low glucose concentration and, as for glycogen synthesis (Figs. 1 and 7*b*), glutamine shifted the glucose response curve towards lower glucose concentrations. The inhibition of ketone-body production may reflect an inhibition of fatty acid oxidation. Therefore the effect of glutamine on lipo-

genesis was studied. Fig. 7(*a*) shows that glutamine stimulated lipogenesis. Acetyl-CoA carboxylase is generally considered as a key regulatory enzyme in lipogenesis. It is an interconvertible enzyme which, like glycogen synthase, is inactivated by phosphorylation and activated by dephosphorylation [39,40]. Its activity is also regulated by citrate, an allosteric stimulator [41]. Changes in metabolite concentration, however, indicated that glutamine decreased the concentration of citrate (Table 2). Therefore, citrate is not involved in the stimulation of carboxylase activity. It is not known whether glutamine could affect the activation state of acetyl-CoA carboxylase.

Conclusion

We have confirmed the observation that glutamine stimulates glycogen synthesis. This effect is particularly striking at low physiological concentrations of glucose, and it results mainly, if not exclusively, from the activation of glycogen synthase. The mechanism involved for synthase activation does not involve a faster inactivation of phosphorylase, as is the case after glucose alone, but it seems to depend on stimulation of synthase phosphatase activity rather than inhibition of synthase kinase. Two functionally distinct proteins have been shown to display synthase phosphatase activity in the liver: a G-component associated with glycogen particles, and a soluble S-component [28,42]. The activity of the G-component, which is strongly inhibited by phosphorylase *a*, is greatly decreased in chronic diabetes [43]. Therefore, we propose that the glutamine-induced activation of synthase results from a stimulation of the activity of the S-component of synthase phosphatase.

The fact that glutamine stimulates both glycogen synthesis and lipogenesis suggests that it could play a more general and anabolic role, which could perhaps extend to the regulation of protein turnover. Not only is glutamine a precursor for protein synthesis, but it also seems to inhibit protein degradation in liver preparations [44–46] and to stimulate protein synthesis in skeletal muscle [47]. This seemingly general anabolic role of glutamine suggests that this amino acid might act through production of an intracellular signal common for the stimulation of glycogen synthesis, lipogenesis and, perhaps, protein synthesis. Our results indicate that, for the stimulation of glycogen synthesis, this putative messenger could be formed directly from glutamine, before the action of glutaminase. An intermediate of the pathway of purine synthesis does not seem to be involved. Other possibilities are glutamine itself, amino sugars, or ionic changes resulting from the Na^+-dependent transport of glutamine [48].

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REFERENCES

1. Seglen, P. O. (1974) Biochim. Biophys. Acta **338**, 317–336
2. Hue, L., Bontemps, F. & Hers, H. G. (1975) Biochem. J. **152**, 105–114
3. Katz, J., Wals, P. A., Golden, S. & Rognstad, R. (1975) Eur. J. Biochem. **60**, 91–101
4. Hems, D. A., Whitton, P. D. & Taylor, E. A. (1972) Biochem. J. **129**, 529–538
5. Walli, A. K., Siebler, G., Zepf, E. & Schimassek, H. (1974) Hoppe-Seyler's Z. Physiol. Chem. **355**, 353–362
6. Whitton, P. D. & Hems, D. A. (1975) Biochem. J. **150**, 153–165
7. Howard, R. B. & Widder, D. J. (1976) Biochem. Biophys. Res. Commun. **68**, 262–269
8. Geelen, M. J. H., Pruden, E. L. & Gibson, D. M. (1977) Life Sci. **20**, 1027–1034
9. Katz, J., Golden, S. & Wals, P. A. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 3433–3437
10. Moser, U. K., Nyfeler, F. & Walter, P. (1977) Experientia **33**, 797
11. Katz, J., Golden, S. & Wals, P. A. (1979) Biochem. J. **180**, 389–402
12. Okajima, F. & Katz, J. (1979) Biochem. Biophys. Res. Commun. **87**, 155–162
13. Boyd, M. E., Albright, E. B., Foster, D. W. & McGarry, J. D. (1981) J. Clin. Invest. **68**, 142–152
14. Chen, K. S. & Lardy, H. A. (1985) J. Biol. Chem. **260**, 14683–14688
15. Rognstad, R. (1985) Biochem. Biophys. Res. Commun. **130**, 229–233
16. Rognstad, R. (1986) Biochem. Arch. **2**, 185–190
17. Hers, H. G. (1976) Annu. Rev. Biochem. **45**, 167–189
18. Stalmans, W. (1976) Curr. Top. Cell. Regul. **11**, 51–97
19. Hue, L., Feliu, J. E. & Hers, H. G. (1978) Biochem. J. **176**, 791–797
20. Kunst, A., Draeger, B. & Ziegenhorn, J. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd edn., vol. 6, pp. 178–185, Verlag Chemie, Weinheim
21. Bontemps, F., Hue, L. & Hers, H. G. (1978) Biochem. J. **174**, 603–611
22. Harris, R. A. (1975) Arch. Biochem. Biophys. **169**, 168–180
23. Hohorst, H. J. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 1st edn., pp. 134–138, Academic Press, New York and London
24. Williamson, D. H. & Mellanby, J. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 2nd edn., pp. 1836–1839, Academic Press, New York and London
25. Mellanby, J. & Williamson, D. H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 2nd edn., pp. 1840–1843, Academic Press, New York and London
26. Hohorst, H. J. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 1st edn., pp. 266–270, Academic Press, New York and London
27. Dagley S. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 2nd edn., pp. 1562–1569, Academic Press, New York and London
28. Doperé, F., Vanstapel, F. & Stalmans, W. (1980) Eur. J. Biochem. **104**, 137–146
29. Golden, S., Wals, P. A., Okajima, F. & Katz, J. (1979) Biochem. J. **182**, 727–734
30. Bollen, M., Hue, L. & Stalmans, W. (1983) Biochem. J. **210**, 783–787
31. Hue, L. (1981) Adv. Enzymol. **52**, 247–331
32. Hers, H. G. & Hue, L. (1983) Annu. Rev. Biochem. **52**, 617–653
33. Zaleski, J., Wilson, D. F. & Erecinska, M. (1986) J. Biol. Chem. **261**, 14082–14090
34. Zaleski, J., Wilson, D. F. & Erecinska, M. (1986) J. Biol. Chem. **261**, 14091–14094
35. Henderson, J. F. & Khoo, M. K. Y. (1965) J. Biol. Chem. **240**, 3104–3109
36. Hershfield, M. S. & Seegmiller, J. E. (1976) J. Biol. Chem. **251**, 7348–7354
37. Mersmann, H. J. & Segal, H. L. (1967) Proc. Natl. Acad. Sci. U.S.A. **58**, 1688–1695
38. Imazu, M., Strickland, W. G., Chrisman, T. D. & Exton, J. H. (1984) J. Biol. Chem. **259**, 1813–1821

39. Carlson, C. A. & Kim, K. H. (1973) J. Biol. Chem. **248**, 378–380
40. Holland, R., Witters, L. A. & Hardie, D. G. (1984) Eur. J. Biochem. **140**, 325–333
41. Hashimoto, T. & Numa, S. (1971) Eur. J. Biochem. **18**, 319–331
42. Stalmans, W., Bollen, M. & Mvumbi, L. (1987) Diabetes/Metab. Rev. **3**, 127–161
43. Bollen, M. & Stalmans, W. (1984) Biochem. J. **217**, 427–434
44. Seglen, P. O., Gordon, P. B. & Poli, A. (1980) Biochim. Biophys. Acta **630**, 103–118
45. Poso, A. R., Schworer, C. M. & Mortimore, G. E. (1982) Biochem. Biophys. Res. Commun. **107**, 1433–1439
46. Seglen, P. O. & Gordon, P. B. (1984) J. Cell Biol. **99**, 435–444
47. MacLennan, P. A., Brown, R. A. & Rennie, M. J. (1987) FEBS Lett. **215**, 187–191
48. Kilberg, M. S., Handlogten, M. E. & Christensen, H. N. (1980) J. Biol. Chem. **255**, 4011–4019

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