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Comparison of the effects of various amino acids on glycogen synthesis, lipogenesis and ketogenesis in isolated rat hepatocytes

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Several amino acids were found to stimulate glycogen synthesis and lipogenesis, and to inhibit ketogenesis in isolated rat hepatocytes. When hepatocytes were incubated in the presence of 20 mM-glucose, the amino acids could be classified in decreasing order of efficiency as follows: glutamine and proline, alanine, aminoisobutyric acid, asparagine and histidine for stimulation of glycogen synthesis; glutamine, proline and alanine for stimulation of lipogenesis; proline and glutamine for inhibition of ketogenesis. The study of the time course revealed that the rates were not linear and were preceded by a lag period. In all conditions studied, glutamine and proline were found to have similar quantitative effects on glycogen synthesis and lipid metabolism. However, their effects differ qualitatively. Indeed, the effects of proline on glycogen synthesis, lipogenesis and glutamate and aspartate content were faster. Moreover, proline increased the hydroxybutyrate/acetoacetate ratio, whereas glutamine did not change it. Incubation of hepatocytes with aminoisobutyric acid or under hypo-osmotic conditions, which increased cell volume and mimicked the amino acid-induced stimulation of glycogen synthesis, had little effect on lipogenesis. In hepatocytes incubated without glucose, ketogenesis was inhibited, in decreasing order of efficiency, by alanine, asparagine, glutamine and proline. Under these conditions, glutamine increased, alanine decreased and asparagine did not affect the concentration of malonyl-CoA. This indicates that the latter cannot be responsible for the inhibition of ketogenesis by alanine and asparagine.

INTRODUCTION

In isolated hepatocytes, glutamine stimulates glycogen synthesis from glucose and gluconeogenic precursors [1–10]. This effect is obtained with physiological concentrations of glutamine and is therefore relevant to the situation *in vivo* [8]. Besides its effect on glycogen synthesis, glutamine is also able to stimulate lipogenesis and to inhibit ketogenesis [8]. On the other hand, glutamine is not the only amino acid known to stimulate glycogen synthesis. Indeed, such a stimulation has been obtained with other amino acids and non-metabolizable amino acid analogues [1,7,9,10]. For example, the effects of glutamine and proline, both of which are transformed in glutamate, are quite comparable [9,10]. More recently, the mechanism of stimulation of glycogen synthesis by glutamine has been reported to be mediated by an increase in cell volume resulting from Na^+-dependent amino acid uptake [9,10], and cell swelling itself has been shown to stimulate glycogen synthesis [9].

The aim of this work was to study (i) whether, like glutamine, other amino acids could stimulate lipogenesis and inhibit ketogenesis, (ii) whether the effect of glutamine on lipogenesis is due to cell swelling, and (iii) whether cell swelling itself could influence lipogenesis and ketogenesis. Since it has been proposed that ketogenesis is controlled by malonyl-CoA [11], we investigated the possibility that this compound is also involved in the control of ketogenesis by amino acids.

MATERIALS AND METHODS

2-Aminoisobutyric acid (AIB; Janssen Chimica), fatty acids (Merck), radiochemicals (Amersham International) and other biochemical reagents (Sigma or Boehringer Mannheim) were purchased as indicated. Bovine albumin (fraction V, from Sigma) was defatted before use [12]. Neutralized fatty acids were bound to albumin as described previously [13].

Hepatocytes were prepared as described previously [14] from overnight-fasted male Wistar rats (200–220 g). The cells (usually 50–70 mg wet wt./ml) were shaken (120 strokes/min) in stoppered scintillation 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 as indicated. All media were in equilibrium with a gas phase of O_2/CO_2 (19:1). Except when stated otherwise, the concentration of amino acids was 10 mM. For the measurement of metabolites, the incubation was stopped by 0.5 M-$HClO_4$. Cell volume was measured as previously described [9].

β-Hydroxybutyrate [16], acetoacetate [17], glutamate [9], aspartate [9], ammonia [18] and urea [19] were measured enzymically in neutralized extracts. Malonyl-CoA was measured by the method of McGarry *et al.* [20]; rat liver fatty acid synthase was purified as described by Linn [21]. Glycogen, lipogenesis and glycogen synthase *a* were measured as indicated in [8].

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

Comparison of the effect of various amino acids on glycogen synthesis, lipogenesis and ketogenesis

The best amino acids found to stimulate glycogen synthesis from 20 mM-glucose were, by decreasing order of efficiency,

Abbreviation used: AIB, aminoisobutyric acid.

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Table 1. Glycogen synthesis, lipogenesis and ketogenesis in hepatocytes incubated with various amino acids or incubated in hypo-osmotic medium

Hepatocytes were incubated with the indicated substrates and 10 mM-amino acids. The results are expressed as percentages (±S.E.M.) of the control values for (*n*) cell preparations. The mean control values are given in square brackets. The rates correspond to the glycogen, lipids or ketone bodies formed between 15 and 75 min. * Significantly different ($P < 0.05$) from the control values.

	Glycogen synthesis	Lipogenesis	Ketone-body production	
Substrate ...	Glucose (20 mM)	Glucose (20 mM)	Glucose (20 mM)	None
Control	100 (14) [1.58 ± 0.25 μmol of glucose/h per g]	100 (16) [0.75 ± 0.11 μmol of ^3H_2O/h per g]	100 (14) [11.72 ± 0.79 μmol/h per g]	100 (9) [39.0 ± 2.1 μmol/h per g]
Glutamine	379 ± 54* (14)	454 ± 75* (16)	80 ± 2* (14)	59 ± 4* (9)
Proline	377 ± 41* (8)	422 ± 137* (10)	75 ± 6* (5)	66 ± 9* (3)
Alanine	242 ± 27* (7)	201 ± 40* (16)	92 ± 5 (7)	40 ± 4* (5)
Asparagine	176 ± 28* (7)	122 ± 32 (9)	89 ± 8 (4)	38 ± 8* (3)
Histidine	150 ± 16* (5)	100 ± 68 (4)	—	83 (2)
NH_4Cl	25 ± 7* (5)	—	115 (2)	45 ± 7* (3)
AIB	200 ± 23* (6)	85 ± 5* (8)	102 ± 4 (5)	104 (2)
Na^+-depleted medium (105 mM-Na^+)	349 ± 67* (7)	158 ± 11* (8)	97 ± 8 (5)	116 ± 2* (4)

glutamine and proline, alanine, asparagine, and histidine ([9] and Table 1). Under these experimental conditions, i.e. in the presence of 20 mM-glucose, the same order of efficiency was found for the ability of these amino acids to stimulate lipogenesis and to inhibit ketogenesis, except that asparagine and histidine were without effect (Table 1). By contrast, in the absence of glucose, ketogenesis was at least 3 times as high as with 20 mM-glucose, and the strongest inhibition of ketogenesis was then obtained with alanine and asparagine, followed by proline and glutamine (Table 1). The anti-ketogenic effect of glucose [22] and alanine [23,24] has previously been described. However, the biochemical reason for the difference in anti-ketogenic capacity of amino acids reported here is not clear. In any case, it is remarkable that proline and glutamine, which were the best stimulators of glycogen synthesis, are also the best for lipogenesis (Table 1), and are known to inhibit protein degradation in liver [25,26].

The time courses of the effects of glutamine, proline and alanine on glycogen synthesis, lipogenesis and ketogenesis in the presence of 20 mM-glucose are compared in Fig. 1. The overall effects of proline and glutamine on glycogen and lipid metabolism were the same when measured over a 75 min incubation period. However, these rates were not linear. Indeed, the stimulation of glycogen synthesis (Fig 1*a*) and lipogenesis (Fig. 1*b*) occurred after a lag period. For glycogen synthesis, this change in rate corresponded to the time-dependent activation of glycogen synthase, which was the fastest with proline, followed by glutamine and alanine (results not shown). By analogy, one could speculate that the lag period occurring in the stimulation of lipogenesis was related to the activation of acetyl-CoA carboxylase, the so-called 'rate-limiting' enzyme for lipogenesis. Such a biphasic phenomenon also appeared in the time-dependent production of ketone bodies (Fig. 1*c*). Indeed, during the first 45 min of incubation, amino acids had no effect on ketogenesis (although a tendency to increase was noted with proline at 15 min), whereas during the last part of the incubation period an inhibition by glutamine and proline was observed. This biphasic effect was less evident in the absence of glucose (results not shown).

From these results, it seems that glutamine and proline affect glycogen and lipid metabolism in a rather similar fashion. However, several differences were observed in their effects. The hydroxybutyrate/acetoacetate ratio measured at 75 min of incubation was differently affected. Whereas glutamine decreased hydroxybutyrate in the same proportion as acetoacetate, proline almost doubled hydroxybutyrate and decreased acetoacetate by 50% (results not shown). This is consistent with the production of reducing equivalents during the first steps of proline metabolism leading to glutamate [27]. In addition, and as mentioned above, the time course indicated that proline exerted the earliest effect. This difference in time course is further illustrated in Fig. 2. The intracellular accumulation of glutamate and aspartate was compared in hepatocytes incubated with glutamine, or proline, or glutamine together with ammonia, a known stimulator of glutaminase [28,29]. Glutamate and aspartate, rather than glutamate alone, were measured, since, as shown by Plomp *et al.* [10], a unique relationship between glycogen synthesis and amino acid concentration was obtained with glutamate and aspartate, but not with glutamate alone. The results show that after 10 min of incubation the glutamate and aspartate content of proline-treated cells was already increased, whereas a similar increase was only observed after 30 min in glutamine-treated cells. As expected, addition of ammonia to glutamine-treated cells abolished the difference at early time points (Fig. 2) and stimulated glycogen synthesis and lipogenesis (Table 2). Addition of 1 mM-ammonia to control cells was not included, because previous studies had shown that it was without effect on glycogen synthesis.

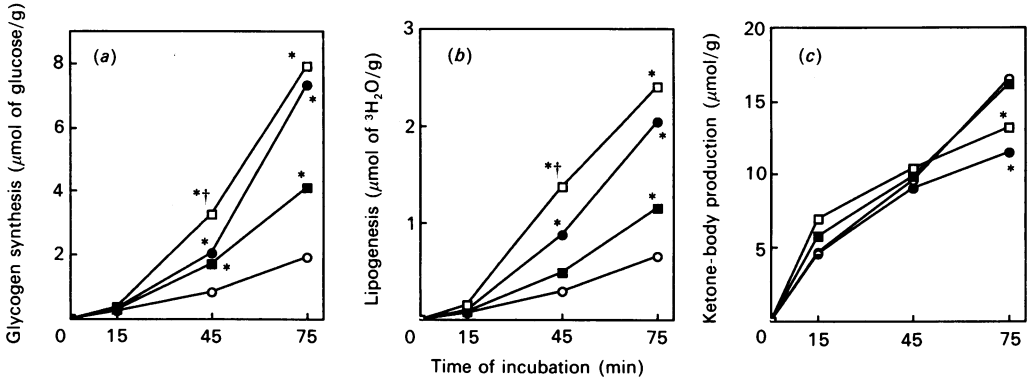

Fig. 1. Time course of glycogen synthesis (*a*), lipogenesis (*b*) and ketogenesis (*c*) in isolated rat hepatocytes

Hepatocytes were incubated with 20 mM-glucose without further addition (○) or with 10 mM-glutamine (●), -proline (□) or -alanine (■) for the indicated periods of time. The values are means for 3 (lipogenesis), 4 (ketogenesis) or 6 (glycogen synthesis) 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.

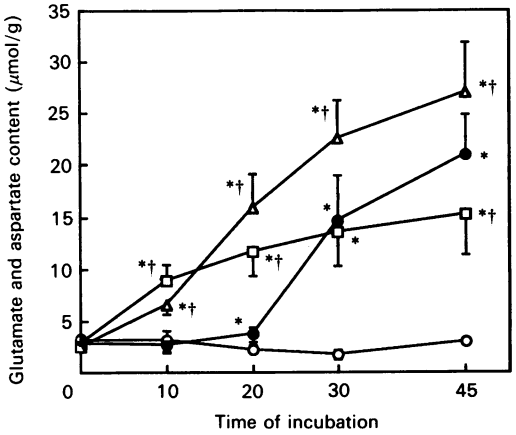

Fig. 2. Time course of the changes in glutamate and aspartate content of hepatocytes

Hepatocytes were incubated with 20 mM-glucose with further addition (○) or with 10 mM-glutamine (●), -proline (□) or -glutamine plus 1 mM-NH_4Cl (△) for the indicated periods of time. The values are means ± S.E.M. for 3 different 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.

Stimulation of lipogenesis and cell swelling

Stimulation of glycogen synthesis by amino acids depends, at least in part, on an increase in hepatocyte volume resulting from amino acid uptake. This interpretation is based on the fact that hepatocyte swelling stimulates glycogen synthesis, and that the extent of stimulation of glycogen synthesis by amino acids was directly proportional to their ability to increase cell volume, except for proline, which stimulated glycogen synthesis more than could be accounted for by the increase in cell volume [9]. A series of experiments were carried out to know whether the stimulation of lipogenesis by amino acids could be related and explained by cell swelling.

Hepatocytes were incubated with AIB, a non-metabolizable amino acid analogue, which is known to increase cell volume [9], and to stimulate glycogen synthesis [7,9]. However, this analogue did not stimulate lipogenesis, and had no effect on ketogenesis (Table 1). On the other hand, no single relationship could be established between cell swelling and stimulation of lipogenesis in hepatocytes incubated with glutamine, alanine or asparagine (Fig. 3*a*). Moreover, when cell volume was increased independently of amino acids by incubating the hepatocytes in hypo-osmotic media, glycogen synthesis was stimulated (4-fold) as expected, whereas lipogenesis was less affected (2-fold increase) (Fig. 3*b*). Finally, the results presented in Table 3 show that the

Table 2. Stimulation by ammonia of glycogen synthesis and lipogenesis in hepatocytes incubated with glutamine

Hepatocytes were incubated with 20 mM-glucose and in the presence of amino acids and ammonia for the indicated periods of time. The values are means ± S.E.M. for three different cell preparations. * Significantly different ($P < 0.05$) from the corresponding control values. † Significantly different ($P < 0.05$) from the corresponding values obtained with glutamine alone.

	Glycogen synthesis (μmol of glucose/g)		Lipogenesis (μmol of ^3H_2O/g)	
	20 min	30 min	20 min	30 min
Control	0.44 ± 0.09	0.47 ± 0.10	0.07 ± 0.01	0.15 ± 0.01
10 mM-glutamine	0.57 ± 0.07	0.98 ± 0.16*	0.10 ± 0.01	0.21 ± 0.03*
10 mM-proline	0.93 ± 0.18*†	1.30 ± 0.28*	0.17 ± 0.02*†	0.37 ± 0.06*†
10 mM-glutamine + 1 mM-NH_4Cl	0.77 ± 0.10*†	1.30 ± 0.21*†	0.12 ± 0.02*†	0.33 ± 0.07*†

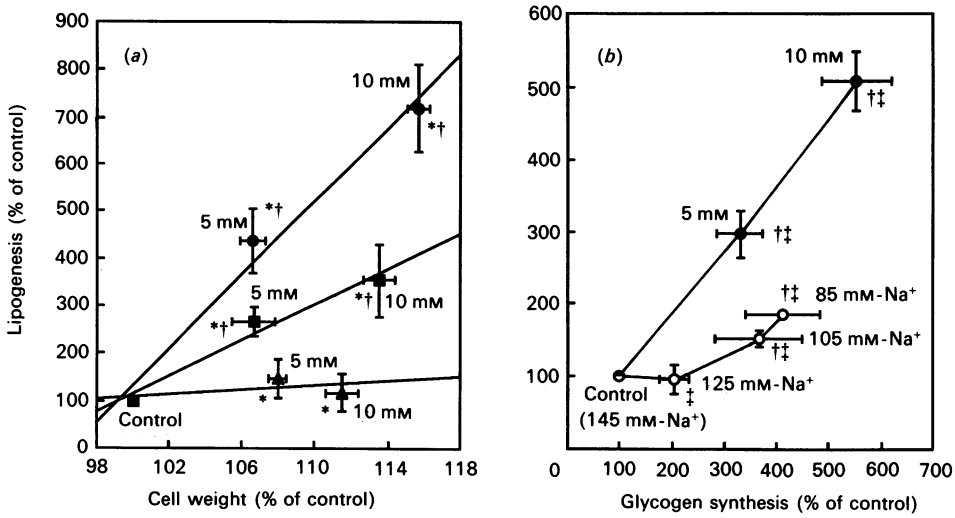

Fig. 3. Relation between cell volume and lipogenesis (*a*) and between glycogen synthesis and lipogenesis (*b*) in isolated rat hepatocytes

Hepatocytes were incubated with 20 mM-glucose and the indicated concentration of glutamine (●), alanine (■) or asparagine (▲) in a standard medium, or in hypo-osmotic Na^+-depleted medium (○). Cell volume was measured after 45 min, and glycogen synthesis and lipogenesis after 60 min of incubation. The values are expressed as percentages (±S.E.M.) of the control values for 4 (*a*) or 3 (*b*) cell preparations. Glycogen synthesis in control was 2.0±1.1 μmol of glucose/h per g. Lipogenesis in control was 0.43±0.16 (*a*) or 0.52±0.07 (*b*) μmol of ^3H_2O/h per g. * Cell volume significantly different ($P < 0.05$) from control values. † Lipogenesis significantly different ($P < 0.05$) from control values. ‡ Glycogen synthesis significantly different ($P < 0.05$) from control values.

Table 3. Additivity of the effects of glutamine and of hypo-osmotic medium on cell volume, glycogen synthesis and lipogenesis in isolated rat hepatocytes

Hepatocytes were incubated with 20 mM-glucose with or without 10 mM-glutamine. Cell volume was measured after 45 min of incubation and glycogen synthesis and lipogenesis after 60 min. The values are expressed as percentages (±S.E.M.) of the control values for (*n*) cell preparations. The mean control values are given in square brackets. * Significantly different ($P < 0.05$) from the control values; † significantly different ($P < 0.05$) from the values in the presence of glutamine.

	Cell volume (% of control)	Glycogen synthesis (% of control)	Lipogenesis (% of control)
Control	100 (4)	100 (11) [1.34±0.19 μmol of glucose/h per g]	100 (5) [0.48±0.06 μmol of ^3H_2O/h per g]
Glutamine	123.3±3.6* (4)	464±66* (11)	547±55* (5)
Na^+-depleted medium (95 mM-Na^+)	112.1±3.6* (4)	312±47*† (11)	192±22*† (5)
Na^+-depleted medium + glutamine (95 mM-Na^+)	142.9±6.6*† (4)	657±91*† (11)	423±67*† (5)

effects of glutamine and of hypo-osmotic medium on both volume changes and glycogen synthesis were additive, whereas those on lipogenesis were not. The results even show that incubation in hypo-osmotic medium antagonized the stimulation of lipogenesis by glutamine (Table 3).

In conclusion, these results indicate that cell swelling has little effect on lipogenesis. Although an increase in cell volume is able to stimulate glycogen synthesis and offers a likely explanation for the glycogenic effect of glutamine, it is, however, not sufficient to explain the lipogenic effect of glutamine.

Mechanism of inhibition of ketogenesis by amino acids

Malonyl-CoA inhibits the carnitine-dependent entry and oxidation of long-chain fatty acids in mitochondria. This is not the case for medium- and short-chain fatty acids [11]. Experiments were carried out to test the possibility that amino acids could interfere with the carnitine-dependent oxidation of fatty acids. Hepatocytes were incubated without glucose, since the inhibitory effect of amino acids was the greatest under this condition. Glutamine, which stimulates lipogenesis and inhibits ketogenesis (Table 1), increased the concentration of malonyl-CoA (Table 4). This increase is consistent with the regulatory role exerted by this metabolite on lipid disposal. On the other hand, both alanine and asparagine inhibit ketogenesis (Table 1) without increasing malonyl-CoA (Table 4); indeed alanine decreased and asparagine had no effect on this metabolite. Moreover, inhibition of ketogenesis by glutamine, alanine and asparagine was obtained whatever the chain length of fatty acids (Table 4). Therefore, inhibition of ketogenesis by these amino acids is in agreement with previous examples of malonyl-CoA-independent control of ketogenesis [30,31].

Addition of 10 mM-NH_4Cl to hepatocytes incubated without glucose was found to inhibit ketogenesis (Table 1). Therefore, we investigated the possibility that inhibition of ketogenesis by amino acids resulted from ammonia production or was inversely related to urea synthesis. Fig. 4(*a*) shows that, in hepatocytes incubated without glucose and with alanine or asparagine, ammonia production was different, although the inhibition of

Table 4. Inhibition of ketogenesis from fatty acids of various chain lengths and concentration of malonyl-CoA in hepatocytes incubated with amino acids

Hepatocytes were incubated for 30 min with the indicated concentration of fatty acids with or without 10 mM-amino acids. The concentration of albumin was 2.5% (w/v). The values are means ± S.E.M. for (*n*) cell preparations. * Significantly different ($P < 0.05$) from the control values.

	Ketogenesis (μmol/30 min per g)			Malonyl-CoA (nmol/g)
Substrate...	0.35 mM-oleate	0.7 mM-octanoate	1.4 mM-butyrate	None
Control	30.3 ± 3.0 (4)	18.7 ± 3.3 (5)	19.4 ± 2.9 (4)	0.59 ± 0.07 (3)
Glutamine	21.2 ± 1.8* (4)	10.7 ± 2.2* (5)	14.5 ± 2.0* (4)	0.83 ± 0.08* (3)
Alanine	9.6 ± 1.3* (4)	5.0 ± 2.7* (5)	10.6 ± 1.7* (4)	0.31 ± 0.06* (3)
Asparagine	10.6 ± 2.7* (4)	7.9 ± 3.6* (5)	9.3 ± 2.1* (4)	0.51 ± 0.10 (3)
Histidine	–	–	–	0.59 ± 0.29 (3)

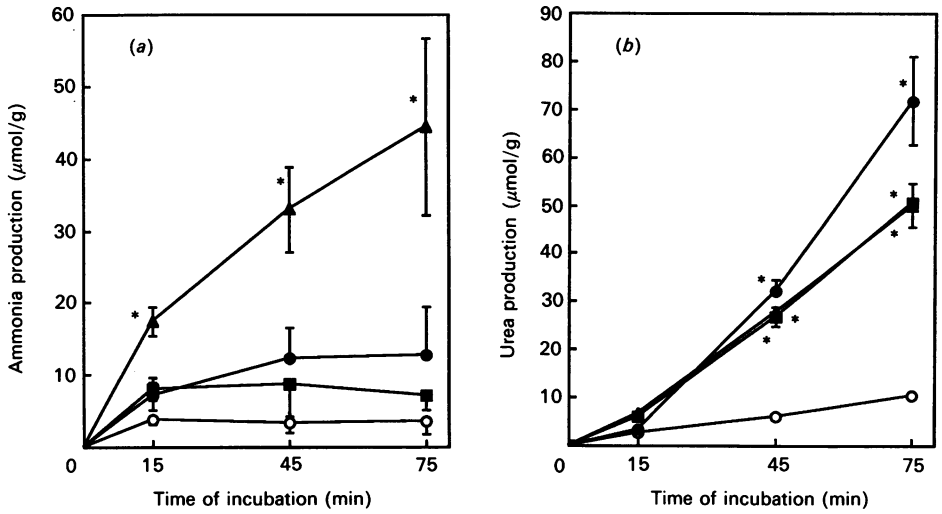

Fig. 4. Ammonia (*a*) and urea (*b*) production in isolated rat hepatocytes

Hepatocytes were incubated for the indicated periods of time without (○) or with 10 mM-glutamine (●), -alanine (■) or -asparagine (▲). The values are means ± S.E.M. for 3 separate cell preparations. * Significantly different ($P < 0.05$) from the corresponding control values.

ketogenesis was the same (see Table 1). In addition, urea production was not inversely related to inhibition of ketogenesis. Indeed, urea production with glutamine was not less than with alanine or asparagine. There was even a tendency to increase with glutamine ($0.1 > P > 0.05$) (Fig. 4*b*). Thus, to explain the inhibition of ketogenesis by these amino acids another mechanism should be invoked, e.g. the availability of oxaloacetate, as previously suggested [30,32].

Conclusion

Glutamine and proline were found to exert similar overall effects on glycogen synthesis, lipogenesis and ketogenesis. The striking similarity between their effects strongly suggests the involvement of a common regulatory mechanism. The relatively slower effect of glutamine on lipid and glycogen metabolism (Fig. 1) could reflect the rather slow metabolism of glutamine by glutaminase (Fig. 2), which is stimulated by ammonia [28,29].

Concerning the common mechanism involved in the control of glycogen and lipid metabolism we can only speculate. An increase in cell volume resulting from amino acid uptake has been proposed to explain, at least in part, the stimulation of glycogen synthesis [9]. Whether cell swelling is also related to stimulation of lipogenesis deserves consideration, although the experimental evidence to support this proposal is rather weak. Indeed, AIB was shown to increase cell volume and to stimulate glycogen synthesis [9], but was without effect on lipogenesis. In addition, no single relationship could be found between cell swelling and stimulation of lipogenesis in hepatocytes incubated with various amino acids. Finally, cell swelling induced by hypo-osmotic media caused less stimulation of lipogenesis than glutamine, although changes in volume and stimulation of glycogen synthesis were quite comparable (Fig. 3*b* and [9]). The interpretation of these data is, however, not simple, since hypo-osmotic media did antagonize the glutamine-induced lipogenesis, and it is not excluded that Na^+ depletion resulting from hypo-osmotic media could interfere with stimulation of lipogenesis.

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