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Control by amino acids of the activity of system A-mediated amino acid transport in isolated rat hepatocytes

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1. The effect of amino acids, in concentrations corresponding to those found in the portal vein of rats given a high-protein diet, was investigated on the activity of system A amino acid transport in hepatocytes from fed rats. 2. Amino acids counteracted the induction of system A by insulin or glucagon. This effect was observed at all concentrations of hormones tested, up to 1 μM. 3. Amino acids did not affect the basal cyclic AMP concentration in hepatocytes, or the large rise in cyclic AMP elicited by glucagon. 4. The reversal of system-A induction was observed at relatively low concentration of amino acids, corresponding to plasma values reported in rats given a basal diet. 5. Amino acids were separately tested: substrates of system A were particularly efficient, but so were glutamine and histidine. Non-metabolizable substrates of system A, such as 2-aminoisobutyrate, were also inhibitory, suggesting that a part of the effect of amino acids is independent of their cellular metabolism. 6. Provision of additional energy substrates such as lactate and oleate did not affect induction of system A or the inhibitory effects of amino acids. Thus amino acids do not act by serving as an energy source and by maintaining the integrity of hepatocytes. 7. Inhibition of mRNA synthesis by actinomycin practically abolished the effect of amino acids on the induction of system A by glucagon. 8. The results suggest that amino acids may promote the synthesis of protein(s) affecting the activity of system A either directly at the carrier unit or at an intermediate stage of its emergence.

INTRODUCTION

Transport of neutral amino acids is regulated by complex processes involving hormonal and adaptive control (Guidotti *et al.*, 1978; Shotwell *et al.*, 1983; Handlogten & Kilberg, 1984). In the liver, the system A-mediated transport [system A was designated according to the terminology defined in Nature (London) (1984) **311**, 308] is induced by both insulin and glucagon (Fehlmann *et al.*, 1979*b*) or by certain physiological situations such as starvation (Fehlmann *et al.*, 1979*a*), diabetes (Samson *et al.*, 1980) or high-protein diets (Fafournoux *et al.*, 1982). *In vitro* it has been established that the transport of amino acids could be affected by cell environment: system A is induced when cells are exposed to conditions of amino acid starvation (Kelley *et al.*, 1978) and declines when cells are provided with amino acids (Handlogten & Kilberg, 1984). Adaptive responses of this type have been described in a variety of tissues (Guidotti *et al.*, 1978), particularly in fibroblasts (Gazzola *et al.*, 1981).

Present knowledge of molecular mechanisms underlying the regulation of amino acid transport is still limited. It is accepted that the induction of system A requires synthesis of both RNA and protein (Pariza *et al.*, 1976; Freychet & Le Cam, 1978). Gazzola *et al.* (1981) have proposed a model for the mechanism of repression and de-repression of system A in fibroblasts, which would involve either synthesis of an inhibitory protein or repression of the carrier synthesis itself. Handlogten & Kilberg (1984) have presented a model in which a putative 'transport-inactivating protein' could be synthesized even after removal of the stimulatory hormone from the medium. The regulation of the synthesis and the turnover rate of such a regulatory protein is still unclear.

Although the activity of system A seems precisely regulated by a variety of factors (hormonal, environmental etc.), there are situations in which cell membrane transport probably constitutes a rate-limiting step in hepatic metabolism of alanine, such as starvation or a high supply of dietary amino acids (Sips *et al.*, 1980; McGivan *et al.*, 1981; Fafournoux *et al.*, 1983). Curiously, such situations are generally accompanied by a noticeable induction of system A.

It was decided to examine further to what extent amino acids themselves could affect the activity of system A (besides classical effects of *cis*- or *trans*-inhibition) or interfere with the effects of other effectors, such as hormones (particularly glucagon and insulin). We report here an inhibitory effect of physiological amounts of amino acids on the induction of system A elicited by glucagon or insulin. Amino acids apparently failed to counteract the effects of glucagon on the intracellular concentration of cyclic AMP. Experiments using an inhibitor of mRNA synthesis (actinomycin D) support the view that amino acids may promote the synthesis of a system-A-inhibitory protein. Furthermore, it appeared that the effects of amino acids could be, to some extent, independent of their further intracellular metabolism.

MATERIALS AND METHODS

Chemicals

2-Amino[^{14}C]isobutyric acid (57 Ci/mol) was purchased from the Commissariat à l'Energie Atomique (Gif-sur-Yvette, France) and the cyclic AMP assay kit from The Radiochemical Centre (Amersham, Bucks., U.K.). Highly purified pig glucagon and pig monocomponent insulin were from Novo (Copenhagen, Denmark). Collagenase, actinomycin D and albumin (fraction V) were obtained from Boehringer (Meylan, France). All

other reagents were of the best grade commercially available.

Preparation of isolated hepatocytes

Hepatocytes were isolated at 14:00 h from rats fed *ad libitum*. Collagenase dissociation was performed by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974). Cell viability estimated by cell-membrane refractoriness in phase-contrast microscopy was 97–99%. Incubations were performed in physiological buffer [Krebs–Henseleit (1932) bicarbonate buffer, pH 7.4] containing 10 mM-Hepes and 2% (w/v) albumin dialysed against the same medium.

Incubation procedures

Experiments were carried out at 37 °C in physiological buffer containing 2% albumin and gassed with CO_2/O_2 (1:19). The vessels were sterilized and the various media were passed through 0.20 μm Millipore filters. Incubations were performed in the presence or absence of hormones and/or amino acids, for various times as indicated in the Figures. The composition of the amino acid mixture is given in the legend of Fig. 1. The composition was designed to mimic the concentration of amino acids measured in the portal vein in rats given a 70%-(w/w)-casein diet (Rémésy, 1982).

Transport experiments

At the required time, samples (300 μl) of hepatocytes (20–30 mg fresh wt. of cells/ml) were taken from the different incubation media. The hepatocytes were centrifuged in 1.5 ml Eppendorf Microfuge tubes for 10 s at about 500 *g* in a swinging rotor. The supernatant was carefully discarded by vacuum aspiration and the cell pellets were resuspended in 1 ml of amino acid-free medium and incubated (generally 10 min) at 37 °C. Thereafter the samples were treated as described above and the cell pellets were suspended in 400 μl of physiological buffer containing 0.2 mM-2-amino[^{14}C]-isobutyrate (^{14}C sp. radioactivity 1.4×10^7 d.p.m./mol). Cells were incubated for 4 min at 37 °C and the reaction was stopped by addition of 1 ml of ice-cold physiological buffer to the Microfuge tubes, which were immediately centrifuged for 5 s at about 1000 *g*: the supernatant was discarded and the cell pellets were resuspended in 1 ml of the same ice-cold buffer. The same procedure was used to collect the cell pellets, which were transferred into counting vials with 3 ml of scintillation liquid (Beckman). The rate of Na^+-dependent transport was determined by subtracting the rate of uptake in the absence of Na^+ (choline Krebs buffer) from that measured in the presence of Na^+ (normal Krebs buffer). The Na^+-free buffer was prepared by replacing NaCl and $NaHCO_3$ with the corresponding choline salts.

Cyclic AMP determination

Hepatocyte suspension (1 ml) was centrifuged (3000 *g*, 5 s) in Microfuge tubes; the supernatant was discarded by vacuum aspiration and cell pellets were rinsed with 1 ml of ice-cold physiological buffer, which was also discarded after centrifugation (3000 *g*, 5 s). The cells were deproteinized with 150 μl of 0.6 M-$HClO_4$; a portion of the $HClO_4$ supernatant was adjusted to about pH 6.8 with K_2CO_3 at 0 °C. Cyclic AMP was determined in the neutralized supernatants with a kit from Amersham. The assay is based on the competition between unlabelled cyclic AMP and a fixed quantity of cyclic [8-^{3}H]AMP for binding to a protein which has a high specificity and affinity for cyclic AMP.

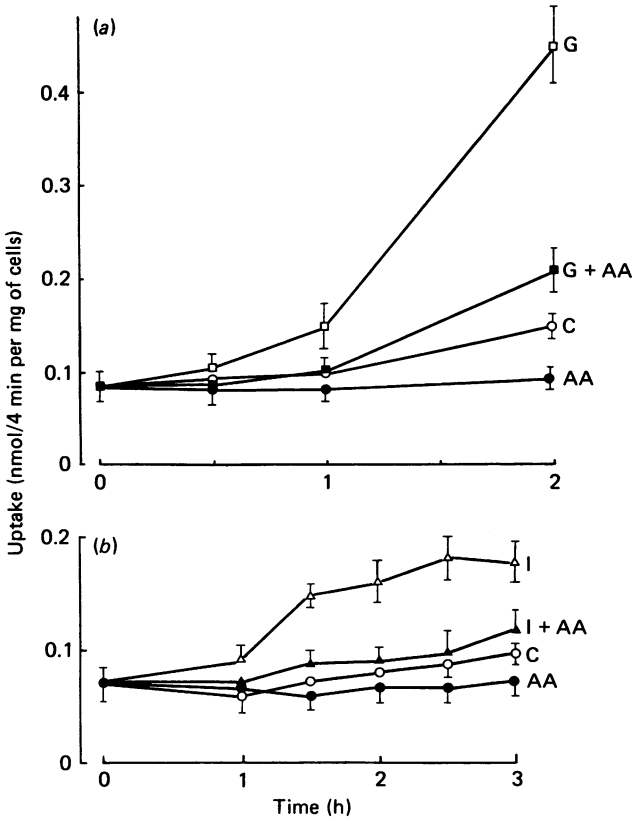

Fig. 1. Effect of amino acids on induction of 2-aminoisobutyrate uptake by glucagon (*a*) or insulin (*b*)

Hepatocytes were incubated in physiological buffer without hormone (○, ●) or containing 0.1 μM-glucagon (□, ■) or 0.1 μM-insulin (△, ▲). ○, □, △, Incubations without amino acids; ●, ■, ▲, incubations in the presence of amino acids in the following concentrations (mM): alanine 2.5, glutamine 2.0, asparagine 0.5, glutamate 0.5, aspartate 0.15, glycine 0.75, serine 1.0, threonine 1.0, proline 2.0, valine 2.5, leucine 1.25, isoleucine 0.62, tyrosine 0.5, phenylalanine 0.5, methionine 0.3, cysteine 0.15, lysine 2.0, histidine 0.4, arginine 0.4, citrulline 0.2, ornithine 0.3, taurine 0.2; tryptophan, 0.02. The results presented are the means ± S.E.M. for three determinations on three batches of cells. Abbreviations: C, control; AA, amino acids; G, glucagon; I, insulin.

RESULTS

Conditions of measurements of 2-aminoisobutyrate transport

The presence of substantial amounts of neutral amino acids in the external medium and in the intracellular compartment may give rise to misleading determinations of 2-aminoisobutyrate transport, if the processes of *cis*- or *trans*-inhibition are not taken into account. The possibility of *cis*-inhibition is easily prevented by dilution of the remaining incubation medium by a much larger volume of Krebs buffer containing 2-amino[^{14}C]isobutyrate. However, amino acids such as alanine may accumulate in the hepatocytes and therefore bring about

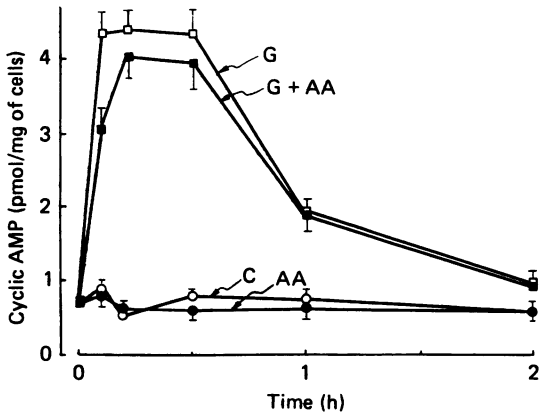

Fig. 2. Effect of glucagon and amino acids on the intracellular concentrations of cyclic AMP

Hepatocytes were incubated in with the concentrations of hormone and amino acids reported in Fig. 1: without (○, ●) or with (□, ■) glucagon; the open symbols correspond to the amino acid-free medium. The results presented are triplicate determinations ±S.E.M. for three batches of cells. For abbreviations see Fig. 1.

trans-inhibition of 2-aminoisobutyrate transport. This point is relevant, since amino acid concentrations are not negligible after 2 h incubation: for example, it was determined that 44% alanine and 90% glutamine still remained in the medium. Other neutral amino acids such as serine and glycine, which are more slowly metabolized than alanine (Rémésy *et al.*, 1983), are likely to remain at physiological concentrations at the end of incubation. The efficiency of a short preincubation (5–10 min) in an amino acid-free medium before measurement of 2-aminoisobutyrate has been studied in the most exacting conditions, namely after 10 min incubation in the presence of the amino acid mixture. In such conditions, intracellular alanine increased to 4.5 mM and, without preincubation, a 15% inhibition of 2-aminoisobutyrate transport was observed. Preincubation of this batch of hepatocytes in an amino acid-free medium for 5 min was sufficient to deplete cellular alanine down to 0.4 mM and for recovery of the basal rate of 2-aminoisobutyrate transport. With non-metabolizable analogues, the rate of disappearance of amino acids from the cell is probably much slower and processes of *trans*-inhibition could become significant. This point has been investigated, after 2 h incubation with 2.5 mM-2-aminoisobutyrate, by measurement of system-A activity after various times of preincubation in amino acid-free medium (from 2 to 40 min). After 15 min, only a 5% *trans*-inhibition was observed. Furthermore, *trans*-inhibition is limited in hepatocytes, since, with the shortest preincubation time (2 min), intracellular 2-aminoisobutyrate was still at 12 mM whereas *trans*-inhibition was only 18%. In fact, Gazzola *et al.* (1980) have reported that in fibroblasts the transport of methyl-2-aminoisobutyrate is poorly sensitive to the process of *trans*-inhibition.

Effect of amino acids on the time course of induction of 2-aminoisobutyrate transport by insulin and glucagon

Figs. 1(*a*) and 1(*b*) show that a noticeable induction of 2-aminoisobutyrate transport was obtained with 0.1 μM-insulin or 0.1 μM-glucagon respectively, of about 250%

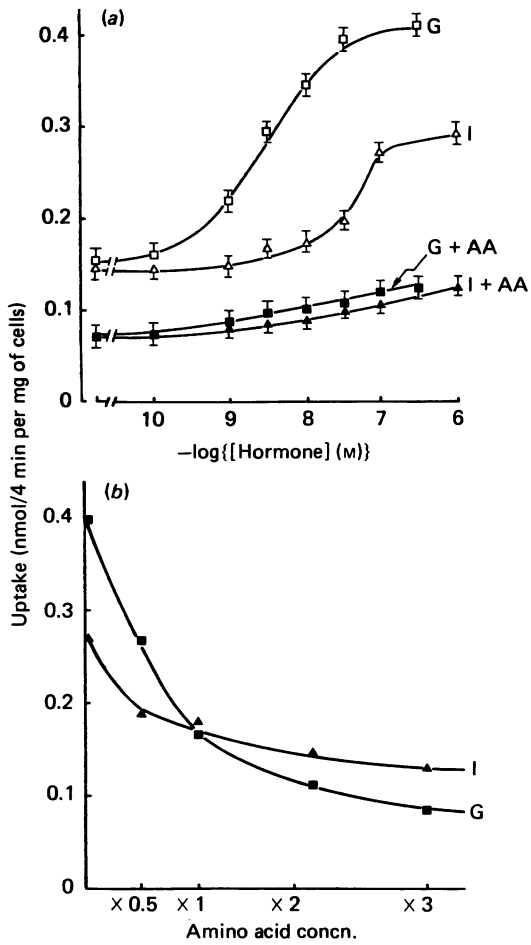

Fig. 3. Dose–response of the effect of amino acids and insulin or glucagon on 2-aminoisobutyrate uptake

(*a*) Effect of amino acids on the dose–response of 2-aminoisobutyrate uptake to insulin or glucagon. Hepatocytes were incubated for 2 h with glucagon (□, ■) or for 2.5 h with insulin (△, ▲), at various concentrations. □, △, Amino acid-free medium; ■, ▲, amino acid-supplemented medium. The results presented are the means ±S.E.M. for three determinations on three batches of cells. (*b*) Dose–response of the effect of amino acids on the hormonal induction of 2-aminoisobutyrate transport. Hepatocytes were incubated for 2 h with 0.1 μM-glucagon (■) or for 2.5 h with 0.1 μM-insulin (▲) in the presence of various concentrations of amino acids, corresponding to 0, 0.5-, 1-, 2- or 3-fold the concentrations of amino acids gives in the legend of Fig. 1. For this experiment, owing to the high concentrations of amino acids utilized, hepatocytes were preincubated in the amino acid-free medium for 15 min instead of 5 min before measurement of 2-aminoisobutyrate transport. The results presented are the means ±S.E.M. for three determinations on two batches of cells. For abbreviations see Fig. 1.

with insulin and up to 600% with glucagon. A slight induction of the carrier was also observed in controls, probably in relation to the lack of amino acids. The presence of amino acids counteracted the induction of 2-aminoisobutyrate transport, whatever the previous treatment. This effect was all the more efficient as the degree of induction was high, as exemplified for glucagon. Nevertheless, the reversal of the induction of 2-

Table 1. Effects of different amino acids on the stimulation of 2-aminoisobutyrate uptake by glucagon

Cells were incubated for 2 h at 37 °C with glucagon (0.1 μM) and with substrates (for composition of amino acids, see legend of Fig. 1). To minimize problems of *trans*-inhibition (particularly with non-metabolizable analogues), the preincubation period in a amino acid-free medium before measurement of 2-aminoisobutyrate transport was extended to 15 min. Each value is the mean of three determinations for two batches of cells.

Substrates	Effects on 2-aminoisobutyrate uptake (% inhibition of induction by glucagon)
Amino acids	68
Lactate (10 mM), pyruvate (1 mM), oleate (1 mM)	0
Amino acids, lactate (10 mM), pyruvate (1 mM), oleate (1 mM)	71
Alanine (2.5 mM)	37
Alanine (5.0 mM)	60
Glycine (2.5 mM)	55
Serine (2.5 mM)	28
Threonine (2.5 mM)	32
Proline (2.5 mM)	57
Glutamine (2.5 mM)	40
Glutamate (2.5 mM)	6
Aspartate (2.5 mM)	3
Leucine (2.5 mM)	0
Phenylalanine (2.5 mM)	25
Methionine (2.5 mM)	34
Ornithine (2.5 mM)	25
Arginine (2.5 mM)	20
Histidine (2.5 mM)	76
Lysine (2.5 mM)	40
2-Aminoisobutyrate (2.5 mM)	41
N-Methyl-2-aminoisobutyrate (2.5 mM)	60

aminoisobutyrate transport was not complete with glucagon, whereas the effects of insulin were more affected. It appears that the regulation of 2-aminoisobutyrate transport by amino acids uniquely involves system A: 20 mM-methyl-2-aminoisobutyrate almost completely abolished the increment of Na^+-dependent 2-aminoisobutyrate transport induced by glucagon, which was responsive to amino acids (results not shown).

Fig. 2 depicts the changes in intracellular cyclic AMP after addition of 0.1 M-glucagon. As expected, this hormone brought about a rapid and considerable increase in cellular cyclic AMP (about 6-fold), which progressively declined after about 30 min. Amino acids did not significantly affect the kinetics of cyclic AMP in control or glucagon-treated cells.

Dose–response of insulin or glucagon stimulation of 2-aminoisobutyrate influx and of its amino acid-induced reversal

To determine the dependence relationships of the hormonal effects on 2-aminoisobutyrate uptake, hepatocytes were exposed to different concentrations of insulin (0.1 nM–1 μM) or of glucagon (0.1 nM–0.5 μM) in either an amino acid-free medium or a medium containing amino acids (Fig. 3*a*). In the absence of hormone, the interval between the batches of cells incubated with or without amino acids reflects the slight induction of system A, which is always observed in amino acid-starved hepatocytes. Amino acids inhibited the induction of 2-aminoisobutyrate transport by insulin or glucagon at all hormone concentrations tested. This inhibition was highly efficient, since it practically abolished the maximal response of 2-aminoisobutyrate transport to insulin or glucagon.

Fig. 3(*b*) shows the dose–response effect of amino acids on insulin and glucagon stimulation of 2-aminoisobutyrate transport. In this experiment, cells were incubated with 0.1 μM-glucagon or -insulin and in the presence of increasing concentrations of amino acids (that reported in Fig. 1 legend, ×0.5, 1, 2 or 3) for 2 h (glucagon) or 2.5 h (insulin). With both hormones, there was a marked decay of 2-aminoisobutyrate-transport induction in the presence of amino acids. The effect was more marked with glucagon, in keeping with its more potent inductive effect. A noticeable effect was still present at the lowest concentration tested, which corresponds to the portal concentration of amino acids in a rat given a 15–20%-(w/w)-protein diet. As reported above, high concentrations of amino acids were very efficient in counteracting the inductive effects of glucagon and insulin.

Specificity of the effect of amino acids on hormonal induction of 2-aminoisobutyrate transport

It was first examined whether the effect of amino acids could be due to the fact that they act as an energy source: for this, incubations were carried out in the presence of additional substrates (lactate, pyruvate and oleate). As shown in Table 1, the supply of energy in the present conditions did not affect the magnitude of induction of 2-aminoisobutyrate uptake by glucagon, nor the inhibitory effects of amino acids on this induction. Glucose was not employed, since it might interfere with the response of cyclid AMP to glucagon (Goldberg & Biava, 1976). Amino acids (branched-chain, neutral, aromatic etc.) were also separately tested (Table 1). It appears that amino acids transported by system A (alanine, serine, glycine and proline) displayed the greatest inhibitory effect, but glutamine and histidine were also very

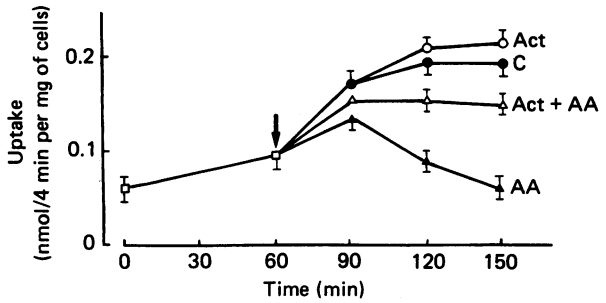

Fig. 4. Effect of actinomycin on the reversal by amino acids of induction of 2-aminoisobutyrate transport by glucagon

Hepatocytes were first incubated for 1 h with 0.1 μM-glucagon. The arrow indicates when cells were washed once with physiological buffer and then incubated in amino acid-free (○, ●) or amino acid-supplemented (△, ▲) medium, with (○, △) or without (●, ▲) actinomycin. The values shown are the means ± S.E.M. of three determinations for two batches of cells. For abbreviations see Fig. 1; Act, actinomycin.

effective. On the contrary, amino acids that are poorly taken up or utilized (glutamate, aspartate and leucine) were practically ineffective. It must be noted that 2-aminoisobutyrate and *N*-methyl-2-aminoisobutyrate also reversed the induction of 2-aminoisobutyrate transport, suggesting that neutral amino acids might operate independently of their intracellular metabolism.

Effect of actinomycin on the decay of 2-aminoisobutyrate transport

Inhibitors such as cycloheximide could allow one to determine whether protein synthesis is involved in the inhibitory effect of amino acids. However, such a treatment will also result in an inhibition of system A induction. Cells were therefore preincubated for 1 h in the presence of glucagon, then the medium containing the hormone was removed and the cells were tested for the amino acid effect in fresh physiological buffer, in the presence or absence of actinomycin (Fig. 4). The process of induction, once initiated, was not interrupted by removal of the hormone and was not inhibited by actinomycin. As expected, amino acids reversed the induction process, and the activity of 2-aminoisobutyrate transport decayed to the initial value within about 90 min. However, addition of actinomycin counteracted the inhibitory effect of amino acids, but this reversal was not complete: this suggests that synthesis of mRNA is involved in the effect of amino acids. In the absence of amino acids, induction of system A was slightly higher after exposure to actinomycin. It has been repeatedly observed that, in amino acid-free medium, the induction of system A after preincubation with glucagon was slightly higher in the presence of actinomycin. This could reflect the presence of small amounts of mRNA coding for inhibitory protein(s) in hepatocytes, in spite of the absence of external amino acids. A parallel experiment carried out with insulin yielded similar results: actinomycin practically abolished the amino acid reversal of hormone-enhanced transport activity (results not shown). For these experiments, it was also observed that exposure of hepatocytes to actinomycin does not affect the overall amino acid utilization, or cellular ATP concentrations.

DISCUSSION

It is now generally accepted that the stimulation of system A depends on synthesis of RNA and protein (Freychet & Le Cam, 1978). According to this view, amino acid deprivation should theoretically decrease overall protein synthesis, but it does not counteract a progressive induction of system A. This suggests that processes involved in the regulation of system A activity might be unaffected by an overall decrease in protein synthesis, all the more since a very minor part of cell protein is probably involved in these processes. An effect of amino acids mediated by a general increase in substrates available for protein synthesis should be ruled out, since the non-metabolizable analogues 2-aminoisobutyrate or *N*-methyl-2-aminoisobutyrate showed effects similar to those of physiological amino acids on system A. In contrast, some essential amino acids were ineffective, even though leucine, for instance, could stimulate protein synthesis and inhibit proteolysis (Pösö *et al.*, 1984).

The present results could also be ascribed to the role of amino acids as an energy source: according to this view, the integrity of hepatocytes incubated without substrates might progressively decline, and leaky cells could mimic induction of 2-aminoisobutyrate transport. However, this explanation seems unlikely, since similar values were observed after 2 h incubation for cell ATP (approx. 2.2 μmol/g wet wt.) and for release of lactate dehydrogenase in the medium, in the presence or absence of amino acids or additional energy fuels such as lactate and oleate. In addition, the fact that non-metabolizable amino acids were also effective calls into question the role of some product of amino acid metabolism. Glutamine and histidine also showed a strong inhibitory effect: these two amino acids are not readily metabolized in hepatocytes from rats fed on a basal diet, but they are substrates for a highly active Na^+-dependent transport system (system N; Kilberg *et al.*, 1980).

Handlogten & Kilberg (1984) have proposed that a transport-inactivating protein may affect the activity of system A: they showed that the mRNA for transport-inactivating protein is relatively long-lived, whereas the protein itself has a half-life of less than 1 h. This is in line with previous observations by Gazzola *et al.* (1981) on cultured human fibroblasts. Whether the present effect of amino acids on the activity of system A pertains to the same process has to be examined further. The effect of actinomycin indicates that synthesis of mRNA is required for the effect of amino acids, but the fact that actinomycin has no effect on induction of system A (once it has been initiated) suggests that mRNA coding for the carrier unit has been already synthesized and that it is relatively long-lived, in agreement with data of Handlogten & Kilberg (1984). However, in the present experiment, the blockade of the decay of system A activity by actinomycin was not complete, which suggests that (i) amino acids may readily act before actinomycin inhibition of mRNA synthesis is fully effective, or (ii) amino acids might promote the synthesis of inhibitory protein(s) from a pre-existing small pool of mRNA. Other factor could also be invoked, such as the existence of several populations of system A units, with respect to the effect of the inhibitory protein(s).

The reversal of system A induction by amino acids is relatively rapid, since, after the incubation medium was

changed, 2-aminoisobutyrate transport started to decrease less than 30 min after addition of amino acids. This lag time might correspond to the time required for synthesis of inhibitory proteins: in this case, there should be (as observed) a transitory period during which the activity of system A is still increasing, concomitantly with synthesis of the inhibitory factor until it reaches a minimal concentration for effective inhibition.

The question arises as to how amino acids may induce the synthesis of proteins which inhibit their transport into the cell, hence autoregulating their own uptake; site A-reactive substrates, even non-metabolizable analogues, were particularly efficient. This seems in line with a previous model (Gazzola *et al.*, 1981) in which the interaction of amino acids with transport proteins would generate a signal promoting the transcription of mRNA coding for the synthesis of an inhibitory factor. It is difficult to assess whether such protein(s) could directly affect the activity of the carrier itself or its synthesis. However, the second hypothesis seems unlikely, assuming that the half-life is about 1.5 h for system A (Handlogten & Kilberg, 1984): the data could hardly account for a practically complete reversal of system A induction within about 1 h, except if the half-life of system A has been overestimated.

The concept of regulatory protein(s) modulating the response of system A activity to hormonal stimuli is still not firmly established. It must be noted that, for the characterization and identification of system A itself, such protein(s) could constitute interesting probes, besides more conventional techniques for studies of membrane proteins. The process of autoregulation of neutral amino acid uptake in hepatocytes could be viewed as a relatively short-term regulation (in the range of 30 min) of amino acid transport. The synthesis of such protein(s) could constitute a mechanism by which the cells limit disturbances (particularly ionic and osmotic) brought about by acute changes in availability of amino acids. Na^+ entry, K^+ exit and cell swelling brought about by transport of neutral amino acids by system A (Bakker-Grünwald, 1983; Fafournoux *et al.*, 1984) could constitute a signal informing some intracellular sensor of the intensity of amino acid transport. This could constitute a relatively sensitive signal, since Kristensen & Folke (1984) pointed out that such effects may be detected down to 0.5 mM external [alanine]. Whether such a process is operative *in vivo* in response to a high availability of amino acids has still to be ascertained.

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