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REGULATION OF AMINO ACID TRANSPORT IN CHICK EMBRYO HEART CELLS

II. ADAPTIVE CONTROL SITES FOR THE "A MEDIATION"

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SUMMARY

The relative roles of transcription and translation of specific mRNA in the adaptive control of amino acid transport by the A mediation in chick embryo heart cells has been investigated, together with the mode of action of interfering amino acids.

Results and conclusions based on the adopted experimental approach include the following:

1. Cells previously incubated in amino acid-free medium containing cycloheximide (phase of inhibited translation) subsequently exhibited a net increase of transport activity when transferred into a plain medium, a medium containing actinomycin D (phase of inhibited transcription), or a medium containing repressive concentrations of alanine (as representative amino acid of the A transport system).

2. The presence of alanine during prior incubation in cycloheximide prevented the increase of activity of the A transport system during subsequent incubation in cycloheximide-free actinomycin D-containing medium. Phenylalanine (as representative amino acid of the L mediation) was ineffective.

3. The synthesis of transport proteins of the A system (as estimated by measurements of transport activity) increased with the duration of cell preincubation in cycloheximide approaching equilibrium after about 40 min. The capacity to synthesize proteins rate-limiting to transport (as a measure of specific mRNA present) decreased during incubation of the cells in cycloheximide when actinomycin D or alanine were added. In both cases capacity decreased as a single exponential with comparable half-lives (approx. 170 min).

4. The degradation of transport proteins of the A mediation followed a single exponential with a half-life of about 200 min when cells were incubated with cycloheximide or puromycin. Actinomycin D did not alter the rate of degradation. Accelerated degradation (half-life of about 80 min) was observed when alanine was added to cells incubated in the presence of cycloheximide.

5. Actinomycin D did not promote the synthesis of transport proteins under conditions of derepressed (absence of added amino acids) or repressed (presence of alanine) transport activity of the A mediation.

6. These observations have been interpreted to indicate that the adaptive regulation of amino acid transport by the A mediation involves a repression–derepression mechanism by amino acids acting at the transcription level coupled, perhaps, to a subsidiary mechanism affecting breakdown (or inactivation) of transport proteins.

INTRODUCTION

In the preceding paper of this series[1] we reported that a time- and amino acid-dependent adaptive system for the transport of a group of neutral amino acids (A mediation)[2] is operative in chick embryo heart cells. Inferences from kinetic studies and investigations with inhibitors of protein and RNA synthesis indicated that the activity of this transport system was regulated by a repression–derepression mechanism by substrate amino acids for which it is competent. However, no firm conclusion has been drawn as to the site(s) of this regulation.

In this report we describe experiments which were intended to define the relative roles of transcription and translation of specific mRNA in the adaptive control of amino acid transport by the A mediation in a muscle tissue. The experimental procedures adopted, involving a temporal separation of the two processes of gene expression by specific inhibitors, are similar to those described in studies by Turner *et al.*[3] on the induction of kynureninase and by Schneider and Wiley[4] on the repression of glucose transport in *Neurospora.*

The results to be presented suggest that mRNA specific for the synthesis of one or more protein(s) needed for amino acid transport by the A mediation was accumulated by the cell under conditions of inhibited translation and that transcription of this mRNA could be repressed by amino acids pertaining to the A transport system. The same amino acids promoted the degradation of transport proteins of the A system during operation.

MATERIALS AND METHODS

The sources for most of the materials used are as listed by Gazzola *et al.*[1]. Cycloheximide, puromycin·2 HCl and actinomycin D were purchased from Nutritional Biochemicals Corporation.

The procedures of cell isolation from chick embryo hearts by collagenase treatment have been described previously in detail[5-7]. Incubations were carried out in silicone-treated glass vessels at 37.5 °C under continuous mild stirring[6] in an atmosphere of O_2–CO_2 (95:5, v/v); the basic incubation medium was Krebs–Ringer bicarbonate buffer containing 8 mM glucose. Under these conditions cardiac cells are viable for several hours[8]. Additions to the medium (amino acids, inhibitors, *etc.*) and washing procedures of the cells before changes in medium composition are specified in Results.

Initial rates of amino acid uptake were measured by transferring samples of cell suspension into conical flasks containing the basic medium with the appropriate additions (labelled amino acid, inhibitors) and incubating the flasks at 37.5 °C for 5 min in a Dubnoff metabolic shaker[1].

The means for determining intracellular accumulation of the tracer amino acid and for evaluating the proper corrections to be introduced were as described by Guidotti *et al.*[6,7].

The rate of protein and RNA synthesis was measured by incubating the cells in the presence of 0.1 mM L-[U-^{14}C]leucine or [5-^{3}H]uridine. Samples of cell suspension were removed at various time intervals and transferred into 5 vol. of 5 % trichloroacetic acid (final concn) containing the corresponding unlabelled compound (1 mM). After standing for 60 min in the cold, the resulting suspensions were rinsed three times with the same solution and heated at 95 °C for 15 min. After centrifugation, the trichloroacetic acid-soluble material was used for measurements of uridine incorporation into RNA; the trichloroacetic acid-precipitable material was collected on fiberglass filters (Whatman, type GFA), washed with chloroform–ether–ethanol (1:2:2, by vol.) and used for measurements of leucine incorporation into protein. Extraction fluids and dried filters were counted in a Packard Tri-Carb scintillation spectrometer.

RESULTS

Time courses of protein and RNA synthesis and the effect of inhibitors

When isolated cardiac cells were incubated in Krebs–Ringer bicarbonate buffer with the sole addition of glucose, the rate of incorporation of labelled leucine into protein was linear over 2 h (Fig. 1); in quantitative terms it was 6.6 μmoles/h per g of cell protein, a value that compares well with those reported previously[6]. A number of inhibitors of protein synthesis (puromycin[9], cycloheximide[10], emetine[11], α,β-dihydroxybutyraldehyde[12], NaF[13]) has been tested at various concentrations for suitability in suppressing translation and in being easily removed from cells upon proper washing procedures. Among them, cycloheximide satisfied both the requirements. As shown in Fig. 1 cycloheximide, added at a concentration of 2 μg/ml, inhibited leucine incorporation into protein by about 90 %. Following removal of the inhibitor, the reduced rate of incorporation of leucine continued for about 20 min, after which the original rate of the uninhibited state was progressively re-established.

The incorporation of labelled uridine into the hot trichloroacetic acid-extractable material from isolated cardiac cells, incubated in Krebs–Ringer bicarbonate buffer supplemented with glucose, is shown in Fig. 2. Actinomycin D (5 μg/ml), added at the beginning or during the incubation, suppressed completely uridine incorporation into RNA. The addition of cycloheximide (2 μg/ml) was without effect on this process.

Temporal separation of transcription and translation of mRNA

As reported previously[1] the activity of amino acid transport by the A system increased with time when isolated cardiac cells were incubated in Krebs–Ringer bicarbonate buffer in the absence of added amino acids. The presence of amino acids pertaining to the A mediation during the incubation period prevented this increase of transport activity and the same result was obtained by adding cycloheximide or actinomycin D[1]. Since RNA synthesis continues after protein synthesis is inhibited by cycloheximide (*cf.* Figs 1 and 2), the capacity of cycloheximide-treated cells to accumulate mRNA coding for proteins involved in amino acid transport might provide a means for investigating separately transcription and translation as a site of regulation on the activity of this process and for elucidating the mode of action of interfering amino acids.

The adopted procedure consisted of a preliminary incubation of isolated cardiac

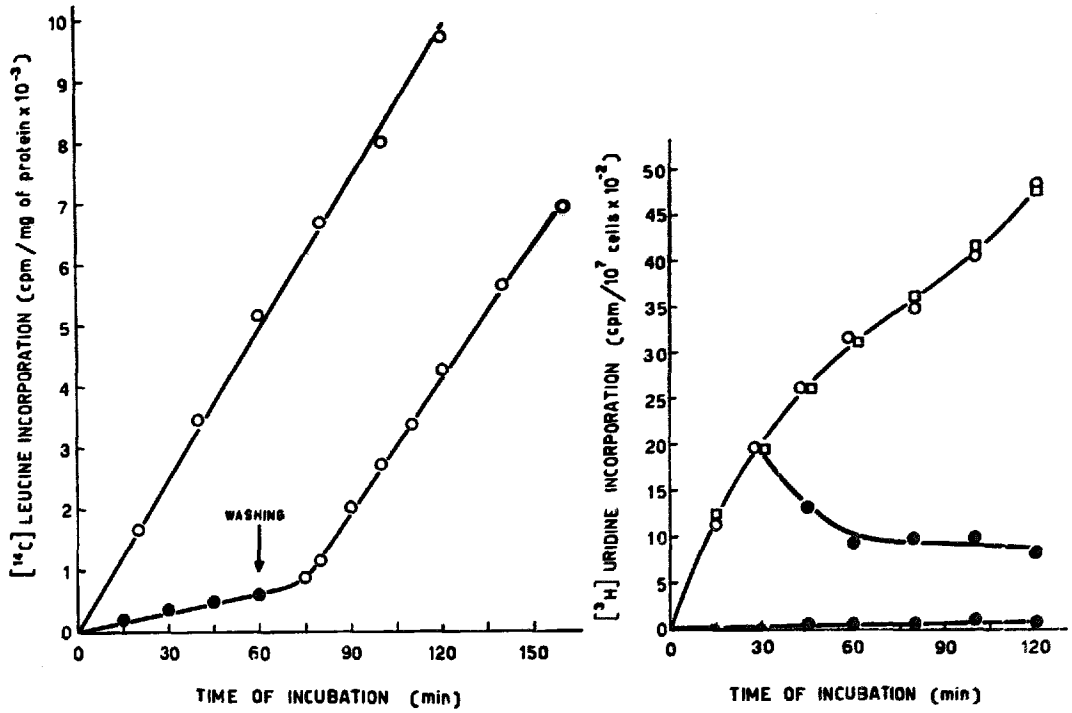

Fig. 1. Time course of labelled leucine incorporation into protein of chick embryo heart cells; effect of cycloheximide. Cells were incubated in Krebs–Ringer buffer containing 0.1 mM L-[U-^{14}C]-leucine in the absence (○) and in the presence (●) of cycloheximide (2 μg/ml). Incubation was at 37.5 °C in an atmosphere of $O_2 + CO_2$ (95:5, v/v). At 60 min the cells incubated with cycloheximide were washed free of the antibiotic. The procedures for protein purification and radioactivity measurement were as described in the text.

Fig. 2. Time course of labelled uridine incorporation into hot trichloroacetic acid-extractable material from chick embryo heart cells; effects of actinomycin D and cycloheximide. Cells were incubated in Krebs–Ringer buffer containing 0.1 mM [5-^{3}H]uridine in the absence (○) and in the presence of actinomycin D (5 μg/ml) (●) or cycloheximide (2 μg/ml) (□). Actinomycin D was added at the beginning or after 30 min of incubation in antibiotic-free medium. Conditions of incubation as in Fig. 1. Experimental details were as described in the text.

cells in the presence of cycloheximide (2 μg/ml) with or without added amino acids (phase of inhibited translation) followed by an incubation period of active or inhibited transcription (by actinomycin D) in the absence or presence of repressive concentrations of amino acids. Appropriate washings insured removal of inhibitor molecules before changes in medium composition and before measurements of amino acid transport activity by the A system. Activity was measured by α-aminoisobutyric acid uptake in experiments of 5-min duration (initial velocity)[1] at various time intervals throughout the incubation.

Fig. 3 shows that isolated cardiac cells, preincubated for 60 min in an amino acid-free medium containing cycloheximide, subsequently exhibited a net increase of transport activity when transferred into a plain medium or into a medium containing actinomycin D. The lag which precedes the appearance of the increase in transport activity is likely to reflect the recovery from cycloheximide treatment (*cf.* Fig. 1) and the length of time required for assembly of nascent transport proteins into the cell membrane. The presence of alanine (5 mM), an amino acid pertaining to the A mediation, during prior incubation in cycloheximide prevented the increase of activity of the A transport system during subsequent incubation in cycloheximide-free actinomycin D-containing medium. Omission of actinomycin D in this phase

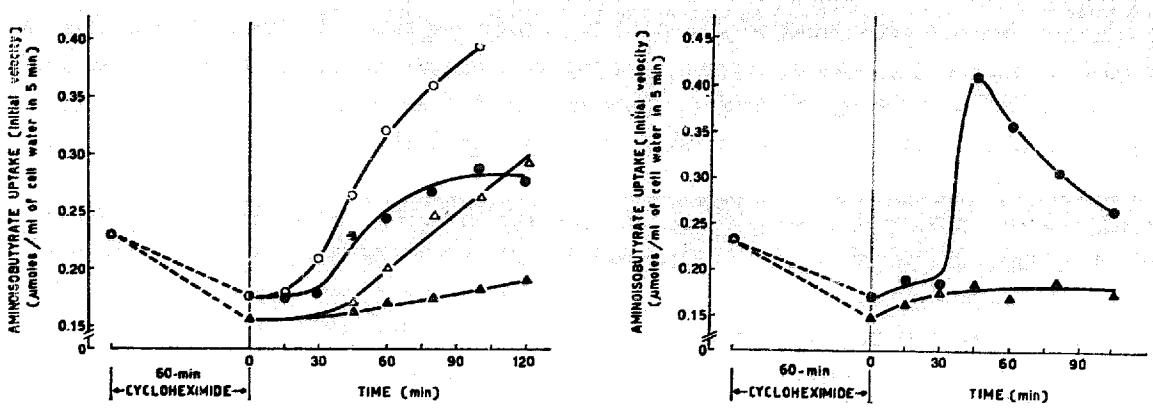

Fig. 3. Changes in amino acid transport activity (A mediation) of cardiac cells incubated in the absence and in the presence of actinomycin D after preincubation in cycloheximide; effect of repressive concentration of amino acid during preincubation. Cells, preincubated for 60 min in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer in the absence (○, ●)or in the presence (△, ▲) of 5 mM L-alanine, were washed, transferred into Krebs–Ringer buffer in the absence (○, △) or in the presence (●, ▲) of actinomycin D (5 μg/ml) and incubated for an additional 120 min. Preincubation and incubation were at 37.5 °C in an atmosphere of O_2+CO_2 (95:5, v/v). Experimental points represent transport activity by the A mediation as measured by α-amino-[1-^{14}C]isobutyric acid uptake (initial velocity) in experiments of 5-min duration at various time intervals throughout the incubation. The procedure for measuring aminoisobutyrate uptake was as described in the text.

Fig. 4. Changes in amino acid transport activity (A mediation) of cardiac cells incubated in the presence of repressive concentration of amino acid after preincubation in cycloheximide; effect of repressive concentration of amino acid during preincubation. Cells, preincubated for 60 min in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer in the absence (○, ●) or in the presence (△, ▲) of 5 mM L-alanine, were washed, transferred into a Krebs–Ringer buffer containing 5 mM L-alanine and incubated for an additional 105 min. Incubations and assay of amino acid transport activity by aminoisobutyrate uptake were as described in the legend of Fig. 3.

allowed a progressive increment of transport activity as expected for cells incubated under derepressed conditions (Fig. 3).

A marked increase of transport activity was also obtained when cells, first incubated in an amino acid-free medium containing cycloheximide, were transferred into a medium containing a repressive concentration (5 mM) of alanine; the presence

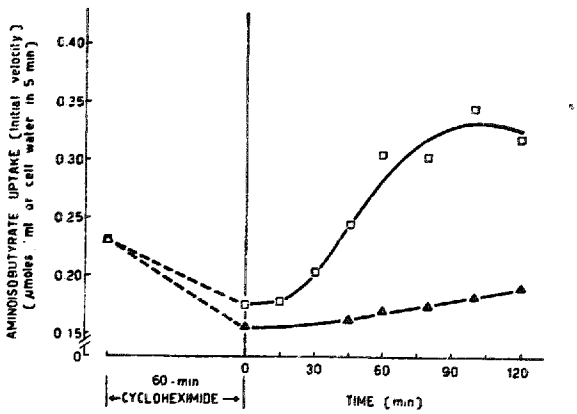

Fig. 5. Changes in amino acid transport activity (A mediation) of cardiac cells incubated in the presence of actinomycin D after preincubation in cycloheximide; effect of amino acids pertaining to the L or A transport systems during preincubation. Cells, preincubated for 60 min in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer in the presence of 5 mM L-phenylalanine (□) or 5 mM L-alanine (▲), were washed, transferred into a Krebs–Ringer buffer containing actinomycin D (5 μg/ml) and incubated for an additional 120 min. Incubations and assay of amino acid transport activity by aminoisobutyrate uptake were as described in the legend of Fig. 3.

of alanine during preincubation in cycloheximide suppressed this increase (Fig. 4). It can be noted that the increase of transport activity observed in the presence of repressive concentrations of amino acids during the second phase (Fig. 4) declined rather rapidly after a maximum reached in 45 min following the initial lag period; on the contrary, the increase of transport activity was more stable with time when transcription during the second phase was inhibited by actinomycin D (Fig. 3). A faster rate of inactivation of the specific transport proteins in the presence of repressive concentrations of amino acid accounts for these findings (*cf.* Fig. 8).

The presence of phenylalanine (5 mM), an amino acid pertaining to the L system, during prior incubation in cycloheximide did not affect the increase of transport activity the the A mediation during subsequent incubation in cycloheximide-free actinomycin D-containing medium (Fig. 5). In contrast, the presence of alanine (A system) during preincubation in cycloheximide suppressed this increase.

Kinetics of mRNA accumulation and degradation during inhibition of translation

The effect of time of preincubation in cycloheximide (2 μg/ml) in the absence of added amino acids on the subsequent increase in activity of amino acid transport by the A mediation is presented in Fig. 6. At various intervals during preincubation, samples of cell suspension were removed, washed free of cycloheximide, and resuspended in a medium containing actinomycin D (5 μg/ml). Activity (aminoisobutyrate uptake in 5-min experiments) was measured at the beginning and at the end of a 60-min period of incubation in the presence of actinomycin D. Its increase was calculated by difference. If transport activity is a measure of the amount of transport proteins present in the system and the amount of proteins resulting from the translation of specific mRNA is proportional to the amount of accumulated message, the results shown in Fig. 6 suggest that the accumulation of mRNA started a few minutes after the transfer of the cells to cycloheximide medium, it increased sharply for 20–30 min and approached a steady-state concentration after about 40 min.

The measurement of the rate of degradation of mRNA accumulated during incubation under conditions of inhibited translation required an experimental

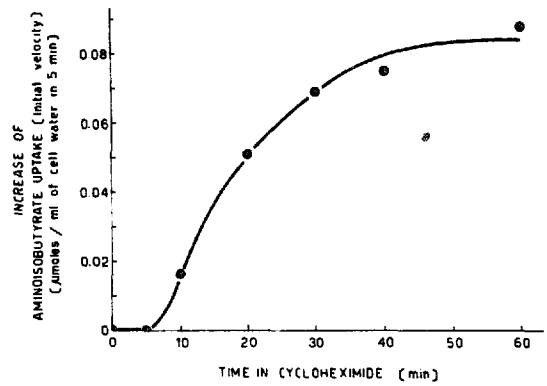

Fig. 6. Changes in amino acid transport activity (A mediation) of cardiac cells as a function of time of preincubation in cycloheximide. Cells were preincubated in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer. At various intervals, samples of cell suspension were removed, washed free of cycloheximide, resuspended in Krebs–Ringer buffer containing actinomycin D (5 μg/ml) and incubated for 60 min. Transport activity (aminoisobutyrate uptake in 5-min experiments) was measured at the beginning and at the end of the incubation in actinomycin D and its increase calculated by difference. Incubations and assay of aminoisobutyrate uptake were as described in the legend of Fig. 3.

procedure in which: mRNA was allowed to accumulate to a maximal concentration by preincubating the cells for 60 min in cycloheximide (*cf.* Fig. 6); the cell suspension was divided in three equal samples which were incubated for an additional 120 min in cycloheximide medium, in cycloheximide-medium containing actinomycin D (5 μg/ml), and in cycloheximide-medium containing 5 mM alanine, respectively; at appropriate intervals, aliquots removed from the three suspensions were washed free of cycloheximide (and of amino acid), resuspended in a medium containing actinomycin D (5 μg/ml) and allowed to translate previously formed mRNA for 60 min; transport activity (aminoisobutyrate uptake in 5-min experiments) was measured at the end of this period. Again, if transport activity is a measure of the amount of transport proteins present in the system, Fig. 7 shows a plot of the maximal capacity to produce transport proteins (a measure of specific mRNA present) as a function of time in cycloheximide. Cells incubated in cycloheximide medium maintained a constant ability to support translation of mRNA for 120 min. The addition of actinomycin D to cells incubated in cycloheximide resulted in a progressive decrease with time of the capacity to synthesize transport proteins and the same result was obtained in the presence of repressive concentrations of alanine. In both cases capacity decreased as a single exponential with comparable half-lives (approx. 170 min). These results suggest that the progressive decrease in the capacity to synthesize transport proteins upon inhibition of mRNA transcription by actinomycin D reflects the breakdown of mRNA (in the presence of cycloheximide) and that amino acids pertaining to to the A mediation suppress the transcription of specific mRNA.

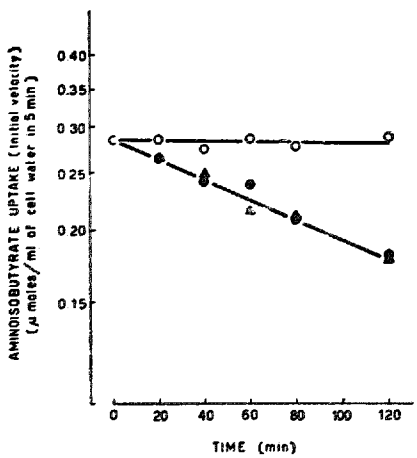

Fig. 7. Changes in the maximal activity of amino acid transport (A mediation), attained by preincubation of cardiac cells in cycloheximide, as a function of time of subsequent incubation in cycloheximide in the absence and in the presence of actinomycin D or repressive concentration of amino acid. Cells were preincubated for 60 min in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer after which they were divided in three equal samples. Samples were incubated for an additional 120 min in the same medium, in cycloheximide medium containing actinomycin D (5μg/ml), and in cycloheximide medium containing 5 mM alanine, respectively. At appropriate intervals, aliquots removed from the three cell suspensions were washed free of cycloheximide (and of amino acid), resuspended in a Krebs-Ringer buffer containing actinomycin D (5 μg/ml), and incubated for 60 min. Transport activity (aminoisobutyrate uptake in 5-min experiments) was measured at the end of this period. Incubations and assay of aminoisobutyrate uptake were as described in the legend of Fig. 3. The data are plotted as a function of time in cycloheximide (○), in cycloheximide *plus* actinomycin D (●) and in cycloheximide *plus* alanine (▲) (note logarithmic scale). In the presence of actinomycin D and alanine, transport activity decreases as a single exponential with a half-life of approximately 170 min.

Kinetics of degradation of transport proteins

An estimation of the rate of degradation of transport proteins (A mediation) has been obtained by measurements of transport activity as a function of time under conditions of inhibited protein synthesis in the presence and absence of added amino acids. Cardiac cell suspensions were preincubated in an amino acid-free medium for 90 min (thus enhancing the activity of the A transport system)[1] after which the selected inhibitor(s) and amino acids were added. Transport activity (as a measure of the amount of transport protein present at any time) was determined every 30 min (for 180 min) by aminoisobutyrate uptake in experiments of 5-min duration (inital velocity) after removal of added compounds. Fig. 8 shows that the activity of the A transport system decreases as a single exponential with a half-life of approximately 200 min when cells were incubated in the presence of cycloheximide or puromycin.

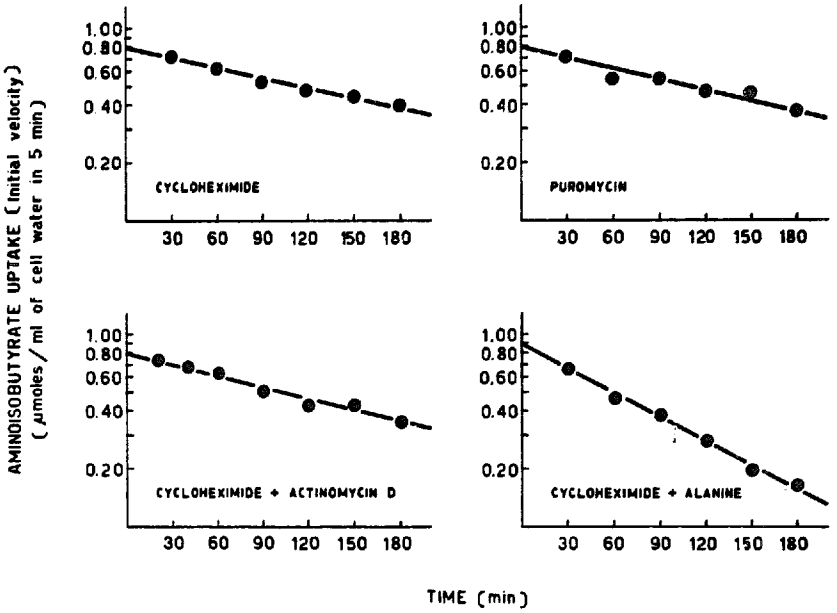

Fig. 8. Changes in amino acid transport activity (A mediation) of cardiac cells incubated under conditions of inhibited protein synthesis after preincubation in uninhibited state; effects of actinomycin D and repressive concentration of amino acid. Cells were preincubated for 90 min in an amino acid-free Krebs–Ringer buffer. After being transferred into Krebs–Ringer buffer containing cycloheximide (2 μg/ml), puromycin (50 μg/ml), cycloheximide *plus* actinomycin D (5 μg/ml), or cycloheximide *plus* 5 mM L-alanine, the cells were incubated for an additional 180 min and transport activity (measured by aminoisobutyrate uptake in 5-min experiments) was followed throughout. Incubations and assay of aminoisobutyrate uptake were as described in the legend of Fig. 3. The data are plotted as a function of time in the different conditions. Note logarithmic scale of the ordinate.

The fact that comparable results are obtained in the presence of inhibitors of protein synthesis acting with different mechanisms[14,15] suggests that the decreasing transport activity reflects the degradation rate of transport proteins. The addition of actinomycin D to cells incubated with cycloheximide did not alter this rate, indicating that the former inhibitor neither prevents nor stimulates transport proteins breakdown. On the contrary, when alanine (5 mM) was added to cells incubated in the presence of cycloheximide, the activity of the A transport system decreased much faster with time (half-life of approx. 80 min) suggesting that the degradation (or inactivation) of specific transport proteins is accelerated during operation. The addition of 5 mM phenylalanine (an amino acid representative of the L system of media-

tion) to cells incubated with cycloheximide did not affect the degradation rate observed in the presence of the inhibitor alone (not shown).

It has been reported that actinomycin D promotes the accumulation of inducible enzymes when given after the induction has taken place. This paradoxical effect called "superinduction"[16], has been ascribed to an inhibition of the enzyme degradation by the antibiotic[17] or to an increase in the rate of enzyme synthesis under conditions of inhibited transcription[16]. As shown in Fig. 8, actinomycin D is not acting by stabilizing transport proteins in our preparation. Fig. 9 indicates that actinomycin D, when added during the phase of derepression (incubation of the cells in amino acid-free medium), abolishes the steady rise of amino acid transport acitivity observed in its absence. This inhibition confirms that derepression is transcription dependent. The slow rate of change in activity of the A transport system after addition of the antibiotic, which does not affect the degradation of transport proteins, is likely to reflect a residual synthesis of these molecules by efficient translation of a rather stable mRNA. When added during a phase of amino acid-mediated repression (incubation of the cells in alanine-containing medium) subsequent to a derepression period, actinomycin D does not alter the rate of change (fast decrease, *cf.* Fig. 8) in activity of the transport system observed in the presence of added alanine. These results indicate that actinomycin D does not promote the synthesis of transport proteins under conditions of derepressed or repressed transport activity and suggest that effects comparable to enzyme "superinduction", or prevention of deinduction, by this inhibitor of RNA synthesis do not occur for the adaptive system of amino acid transport (A mediation) in chick embryo heart cells.

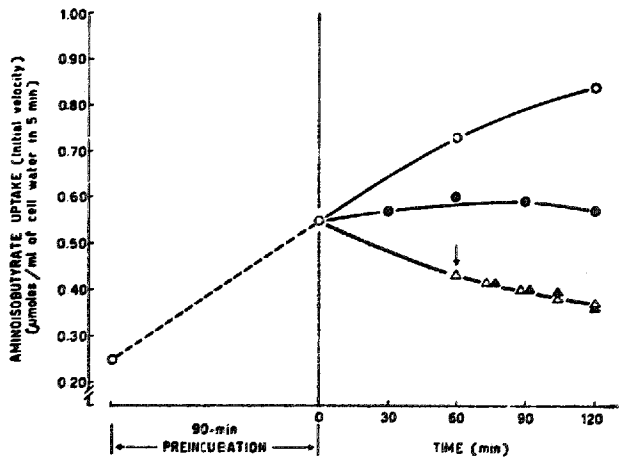

Fig. 9. Changes in amino acid transport activity (A mediation) of cardiac cells incubated in the presence of actinomycin D under derepressed (absence of added amino acids) or repressed (presence of added alanine) conditions. Cells were preincubated for 90 min in an amino acid-free Krebs–Ringer buffer and subsequently incubated for an additional 120 min in the same medium in the absence (○) and in the presence of actinomycin D (5 μg/ml) (●), of 5 mM L-alanine (△), or of 5 mM L-alanine *plus* actinomycin D (added after 60 min of incubation, arrow and ▲). Transport activity (aminoisobutyrate uptake in 5-min experiments) was measured at various intervals throughout the incubations. Incubations and assay of aminoisobutyrate uptake were as described in the legend of Fig. 3.

DISCUSSION

The transport of amino acids in chick embryo heart cells is performed by specific systems of mediation acting on discrete groups of substrate molecules[1]. Among

these transport agencies, the A system[2] varies in its activity being subject to regulation by amino acids for which it is competent[1]. Adaptive control of amino acid transport by the A mediation has been found to occur in various muscle tissues (heart, diaphragm, skeletal muscles) from mammalian (rat, mouse) and avian (chicken) sources (unpublished results). Recently, a time-dependent control mechanism for amino acid transport by the A system has been described in immature rat uterus[18]. These observations render feasible the concept that adaptive regulation is an essential property for the modulation of amino acid transport in muscle tissues. The experimental approach described in this paper has attempted to define the site(s) of this adaptive control mechanism.

It is of course recognized that most of the conclusions presented below are based on indirect evidence which leans on the validity of a number of assumptions, such as: that the inhibitors applied are specific as to site and action, that transport activity is a measure of the amount of transport proteins present in the system, that changes in the concentration of transport proteins are proportional to the amount of specific mRNA accumulated in the cell.

With these limitations, the simplest interpretation of the results presented in Figs 3, 4 and 5, based directly on the Jacob–Monod model[19], favours a repression–derepression control mechanism operative at gene transcription. Amino acids transported by the A system would effect a selective change at this level (perhaps by interaction with a repressor molecule), resulting in a decreased synthesis of transport protein-specific mRNA and thereby in a reduced synthesis of protein components of the A system. If this is the case, the explanation of the experimental data is the following: (a) mRNA coding for one or more proteins involved in amino acid transport by the A mediation is synthesized during cell incubation in cycloheximide-containing medium when amino acids are omitted; (b) this mRNA is translated during subsequent incubation in cycloheximide-free medium under conditions of active or inhibited transcription; (c) the presence of amino acids pertaining to the A mediation prevents the synthesis of the specific mRNA during cell incubation in cycloheximide-containing medium and no substantial increase of activity of the A transport system can be detected in subsequent incubation in cycloheximide-free medium under conditions of inhibited transcription. Regulation of enzyme induction in eucariotes by mechanisms acting at the transcription level has been reported by Nebert and Gelboin[20] for hydrocarbon hydroxylase, by Turner *et al.*[3] for kynureninase and by Lee *et al.*[21] for tyrosine transaminase. Horowitz *et al.*[22] have reported that repression of tyrosinase synthesis by amino acids in *Neurospora* occurs *via* an unstable repressor protein acting at gene transcription. Repression of glucose transport (System II) in *Neurospora* by high levels of glucose apparently occurs at the level of transcription[4].

The results shown in Figs 3, 4 and 5, however, can also be interpreted on the basis of the inhibition of mRNA translation by a labile repressor capable of promoting mRNA degradation[16]. If this post-transcriptional repressor is activated in the presence of amino acids pertaining to the A mediation and is inactivated (or degraded) in their absence, the explanation of the experimental data is the following: (a) cell incubation in cycloheximide-containing amino acid-free medium results in the accumulation of transport protein–mRNA and repressor–mRNA and in the inactivation of the initially present repressor; (b) both mRNAs are translated during subsequent incubation in

cycloheximide-free medium under conditions of active or inhibited transcription, but the newly formed repressor is inactive (absence of amino acids); (c) the addition of amino acids pertaining to the A mediation during prior incubation in cycloheximide maintains the initially present repressor in an active state and, when present during the translation phase, activates the repressor which is being translated; thus, under the latter condition no increase of activity of the transport system can be detected. That enzyme induction and repression in eucaryotes can be regulated at a post-transcriptional level has been proposed by Tomkins and coworkers[16,23] for tyrosine transaminase and by Alescio *et al.*[24] for glutamine synthetase. Post-transcriptional control of protein synthesis has also been suggested for a variety of eucaryotic cells[25-28].

At this point both the presented interpretations might be equally valid. Some additional results, however, are better explained by postulating a mechanism of regulation acting at the transcription level: (a) a strong argument in favour of the occurrence of a post-transcriptional regulation of enzyme induction is that inhibitors of RNA synthesis, such as actinomycin D, are capable of reactivating enzyme synthesis after deinduction has begun[16], possibly by suppressing repressor synthesis (and allowing the repressor to decay); the enzyme-specific mRNA would then dissociate from the repressor resulting in the formation of active mRNA with return of enzyme synthesis; our results (*cf.* Fig. 9) are inconsistent with this observation: reactivation (or derepression) effects are not detected in chick embryo heart cells upon addition of actinomycin D under conditions of amino acid-mediated repression of transport activity; (b) the addition of amino acids pertaining to the A mediation to a cycloheximide-containing medium progressively decreases the maximal amount of previously accumulated mRNA (as estimated by the capacity to synthesize a protein rate limiting to transport which, in turn, is measured by transport activity; *cf.* Fig. 7); this observation can be interpreted to indicate the occurrence of mRNA degradation (revealed by the inhibition of its synthesis) rather than activation of a post-transcriptional repressor whose synthesis is prevented by the inhibition of translation (presence of cycloheximide). Hence, a regulation by amino acids acting at gene transcription is favoured. Further support to this interpretation is provided by the finding (*cf.* Fig. 7) that the rate of mRNA degradation after addition of amino acids is identical to that found in the presence of actinomycin D. Since both experiments were performed in the presence of cycloheximide, which is likely to protect mRNA from degradation[4], no inferences can be drawn as to the true rate of mRNA decay under conditions of active translation.

The increased degradation (or inactivation) of transport proteins of the A system in the presence of high concentrations of amino acids taken up by the same agency (*cf.* Fig. 8) suggests the occurrence of an additional control mechanism at the transport site. The phenomenon is specific since amino acids pertaining to the L mediation are ineffective. Perhaps, operation of the transport system and breakdown (or inactivation) of the constituent molecules are strictly coupled processes and the adaptive regulation of amino acid transport by the A mediation requires two different sites. If this were the case, the cell would respond: (a) with an increased synthesis of transport proteins — dependent on derepression of specific mRNA transcription — when exposed to conditions of amino acid shortage and (b) with an enhanced breakdown of transport proteins accompanying the progressive suppression

of their synthesis — the latter dependent on repression of mRNA transcription — when matched by conditions of increased amino acid supply.

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