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REGULATION OF AMINO ACID TRANSPORT IN CHICK EMBRYO HEART CELLS

IV. SITE AND MECHANISM OF INSULIN ACTION

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SUMMARY

The regulation of amino acid transport by insulin in chick embryo heart cells has been studied. Experiments were designed to identify the system(s) of mediation involved in this regulation and to investigate the mechanisms of the hormone action.

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

1. Among the four systems (A, ASC, L, Ly^+) found to be operative in cardiac cells for the transport of neutral and basic amino acids (Gazzola, G. C., Franchi-Gazzola, R., Ronchi, P. and Guidotti, G. G. (1973) Biochim. Biophys. Acta 311, 292–301), only the A mediation is responsive to insulin.

2. The hormone enhances the activity of this mediation by increasing the maximal velocity (V) of transport (without substantial changes in K_m) even under conditions of inhibited protein synthesis.

3. The rate of degradation of protein components of the A mediation (as estimated by measurements of transport activity under conditions of inhibited protein synthesis) is decreased when cells are incubated in the presence of insulin. The hormone does not affect this rate for systems ASC, L and Ly^+.

4. Cells previously incubated in a medium containing cycloheximide (phase of inhibited translation) subsequently exhibit a net increase of transport activity (A mediation) when transferred into a medium containing actinomycin D (phase of inhibited transcription). The presence of insulin during pre-incubation in cycloheximide has no effect on the subsequent rate of change in transport activity; when added to the second phase, the hormone definitely enhances transport activity.

5. These observations have been interpreted to indicate that insulin modulates the activity of the A transport system by acting at two different sites: at the cell membrane by protecting specific transport proteins against degradation and at a post-transcriptional level by increasing the rate of synthesis of these transport proteins.

Abbreviations: BCH, 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid; MeAIB, α-(methylamino)isobutyric acid; GPA, 4-amino-1-guanylpiperidine-4-carboxylic acid.

INTRODUCTION

Insulin enhances the accumulation by muscle of a number of amino acids [1, 2]. It has been suggested that this hormonal effect is restricted to substrates of Transport System A [3]. That this is the case in isolated chick embryo heart cells has been anticipated in previous studies [4]. The amino acids that responded to insulin with a marked increase of uptake corresponded to those assigned to the A mediation [5, 6], an adaptive transport system subject to regulation by its substrate molecules [7].

Kinetic studies with representative amino acids, under experimental conditions in which results approximated initial rates of entry [8, 9] or steady-state values of intracellular to extracellular distribution [10, 11], indicated that insulin promotes the intracellular accumulation of these molecules into the heart tissue by accelerating the maximal velocity of transport without substantial changes in substrate concentration for half-maximal transport velocity. These results suggested that the hormone increased the availability of active transport molecules at the cell membrane [4]. In turn, an enhanced availability in the constituents of a transport agency might result from either stimulation of their synthesis or a decreased rate of their degradation.

The purpose of this work was to investigate the sites of insulin regulation of amino acid transport across the membrane in chick embryo heart cells. Preliminarly, we have identified the A mediation as the transport agency which undergoes a control of its activity by the hormone. Then we studied insulin actions on: (a) the kinetic parameters of amino acid transport under conditions of inhibited protein synthesis; (b) the rate of degradation of protein components of the transport systems; (c) the transcription and translation of mRNA coding for protein(s) involved in amino acid transport.

The results to be presented indicate that insulin acts at two different sites: at the cell membrane by protecting protein components of transport system A against inactivation, and at the ribosomal level by increasing the rate of translation of specific mRNAs coding for the synthesis of transport proteins.

MATERIALS AND METHODS

The sources for most of the materials used are as listed in the preceding papers of this series [6, 7, 12]. α-(Methylamino)isobutyric acid (MeAIB), 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid (BCH; isomeric form b(±)) and 4-amino-1-guanyl-piperidine-4-carboxylic acid (GPA) were gifts from Dr Halvor N. Christensen (University of Michigan, Mich., U.S.A.). Bovine insulin (Lilly, Batch no. PJ4609, 23.8 units/mg) was kindly supplied by Dr Mary Root (Eli Lilly Research Laboratories, Indianapolis, Ind., U.S.A.).

The procedures of isolation of cells from chick embryo heart by collagenase treatment have been described in detail previously [1, 8]. Incubations were carried out in silicone-treated glass vessels at 37.5 °C under continuous mild stirring [1] 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 [10]. Additions to the medium (amino acids, in-

hibitors, insulin) 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 the cell suspension into flasks containing the basic medium with the appropriate additions (labelled amino acid, inhibitors, insulin) and incubating the flask at 37.5 °C for 5 min in a Dubnoff metabolic shaker [6].

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. [1, 9].

RESULTS

Insulin affects Transport System A

The results presented in Table I indicate that insulin accelerates the uptake of the naturally occurring amino acids glycine, L-proline, L-alanine and L-serine and of the analogues α-aminoisobutyric acid and cycloleucine; the hormonal effect ranges between 42 % for α-aminoisobutyric acid and 16 % for serine, being always statistically significant ($P < 0.001$). All these amino acids belong to the group taken up, primarily or appreciably, by the A transport system [6, 13] and undergo adaptive

TABLE I

AMINO ACID UPTAKE BY ISOLATED CARDIAC CELLS: CHANGES IN TRANSPORT ACTIVITY WITH TIME AND EFFECT OF INSULIN

Cardiac cell suspensions were incubated for 2 h in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 8 mM glucose. Incubation was at 37.5 °C in an atmosphere of O_2–CO_2 (95 : 5, v/v). When present, insulin was 0.2 unit/ml. Amino acid uptake (initial velocity) was measured by transferring samples of cell suspension (10^7 cells) into flasks containing Krebs–Ringer bicarbonate supplemented with 8 mM glucose and the ^{14}C-labelled amino acid (0.1 mM, final concn) under study and incubating for 5 min at 37.5 °C. Values are means ±S.E. for the number of experiments shown in parentheses. n.s., not significant, that is, $P > 0.05$.

Amino acid	Uptake (μmoles/ml of cell water in 5 min)			*P* for insulin stimulation
	Incubation time (h)			
	0	2 (No insulin)	2 (Insulin)	
Glycine (9)	0.27±0.02	0.40±0.03	0.48±0.04	< 0.001
L-Proline (10)	0.44±0.04	0.87±0.06	1.21±0.07	< 0.001
L-Alanine (10)	1.03±0.07	1.66±0.09	2.18±0.13	< 0.001
L- Serine (10)	0.68±0.05	1.11±0.07	1.29±0.06	< 0.001
α-Aminoisobutyric acid (11)	0.31±0.03	0.74±0.08	1.05±0.11	< 0.001
Cycloleucine (8)	0.59±0.05	0.73±0.05	0.92±0.07	< 0.001
L-Threonine (10)	0.62±0.04	0.43±0.02	0.49±0.03	< 0.05
L-Methionine (9)	0.22±0.02	0.17±0.02	0.17±0.02	n.s.
L-Phenylalanine (7)	0.25±0.02	0.20±0.02	0.22±0.03	n.s.
L-Valine (7)	0.38±0.04	0.23±0.03	0.22±0.02	n.s.
L-Leucine (9)	0.58±0.03	0.29±0.02	0.28±0.02	n.s.
L-Lysine (7)	0.71±0.05	0.30±0.03	0.28±0.03	n.s.
L-Histidine (3)	0.56±0.08	0.36±0.07	0.38±0.05	n.s.

regulation [6, 12]. Only minor changes occur with L-methionine, L-phenylalanine, L-valine, L-leucine, L-lysine and L-histidine which are preferential substrates of Transport Systems L and Ly^+ [6, 13]; both Systems L and Ly^+ do not exhibit a time-dependent increase of transport activity [6, 12]. Intermediate behaviour is observed with L-threonine (Table I), a good substrate of the ASC system in several tissues [14, 15]; this amino acid does not participate in adaptive regulation [6], whereas its uptake is apparently accelerated by insulin ($P < 0.05$).

These results identify the A mediation as an insulin-sensitive transport system and suggest that the ASC system might also be involved in this hormonal regulation. To gain information on the latter point the effect of insulin on transport activity by the ASC system has been investigated. Using L-alanine, L-serine and L-threonine (which are known to enter the cell by Systems A, ASC and, less efficiently, by system L, cf. ref. 12) as tracer amino acids in the presence of sufficient MeAIB and L-phenylalanine to prevent amino acid uptake by Systems A and L, transport activity of the ASC system can be operationally defined (within the limits of the inhibition analysis carried out with these substrates). Results shown in Table II indicate that insulin does not affect transport activity of this mediation, provided that amino acid uptake by the A system is fully prevented. The lack of insulin effect on the rate of degradation of protein components of Transport System ASC (cf. Fig. 3) confirms this conclusion. Therefore, the hormonal stimulation of threonine uptake reported in Table I is likely to reflect the effect of insulin on the A mediation (whose activity progressively increases with time under derepressive conditions, cf. refs 6 and 12), through which a definite fraction of this amino acid can be taken up. In agreement, the differential acceleration by insulin of the uptake for the various amino acids as reported in Table I appears to be positively correlated to the fraction of their total uptake which

TABLE II

AMINO ACID UPTAKE BY THE ASC TRANSPORT SYSTEM IN ISOLATED CARDIAC CELLS: EFFECT OF INSULIN

Cardiac cell suspensions were incubated for 2 h in Krebs–Ringer bicarbonate buffer containing 8 mM glucose in the presence and absence of insulin (0.2 unit/ml). Incubation was at 37.5 °C in an atmosphere of O_2–CO_2 (95 : 5, v/v). Amino acid uptake (initial velocity) was measured by transferring samples of cell suspension (10^7 cells) into flasks containing the same medium supplemented with 0.1 mM L-[U-^{14}C]alanine, L-[U-^{14}C]serine or L-[U-^{14}C]threonine and unlabelled α-(methylamino)isobutyric acid at the specified concentrations, and incubating for 5 min at 37.5 °C. 5 mM L-phenylalanine was also present during uptake measurement (to prevent amino acid uptake by the L system [6]). Each value is the mean of 4 separate determinations.

MeAIB added during uptake measurement (concn, mM)	Uptake (μmoles/ml cell water in 5 min)								
	L-alanine			L-serine			L-threonine		
	Insulin		Stimulation (%)	Insulin		Stimulation (%)	Insulin		Stimulation (%)
	−	+		−	+		−	+	
2.5	0.50	0.59	18	—	—	—	0.45	0.49	9
5	0.44	0.48	9	0.37	0.38	3	0.44	0.45	2
10	0.40	0.38	0	0.35	0.34	0	0.41	0.41	0
20	0.34	0.31	0	—	—	—	0.34	0.34	0

occurs through a MeAIB-inhibitable system, assumed to represent the A mediation (correlation coefficient 0.95; Fig. 1). Evidence for an insulin-insensitive, yet Na^+-dependent, component of alanine uptake (ASC system) into the isolated rat diaphragm has been reported by Riggs and McKirahan [16].

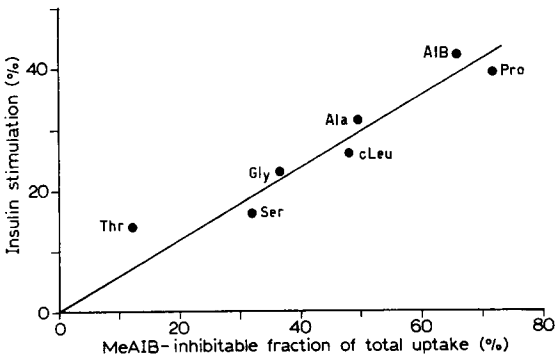

Fig. 1. Insulin stimulation versus the MeAIB-inhibitable fraction of total uptake for insulin-responsive amino acids. Insulin effect (expressed as percentage increment of transport activity after 2 h of incubation of isolated cardiac cells in the presence and absence of the hormone) has been calculated from data given in Table I. The MeAIB-inhibitable fraction of amino acids uptake has been determined in experiments of 5-min duration incubating the cells with the labelled amino acid under study in the absence (total uptake) and presence of unlabelled α-methylaminoisobutyric acid (residual uptake) at concentrations ranging between 5 and 10 mM (to prevent amino acid uptake by Transport System A, cf. Table II). Transport activity of the MeAIB-inhibitable component (assumed to represent system A) has been calculated from the difference between total and residual uptake, and expressed as percentage of total uptake.

Kinetics of amino acid transport under conditions of inhibited protein synthesis

As reported previously, insulin stimulation of amino acid uptake by isolated cardiac cells operates through a mechanism affecting the maximum velocity of transport [9, 10] and remains effective for a few hours after suppression of protein and RNA synthesis by cycloheximide, puromycin and actinomycin D [4].

Using α-aminoisobutyric acid as a representative substrate of the A mediation we have now investigated whether the kinetic action of the hormone on amino acid transport is still consistent with an increase of V under conditions of inhibited protein synthesis. The adopted procedure required a preliminary incubation (30 min) of isolated cardiac cells in the presence of cycloheximide (2 μg/ml) followed by a 60 min incubation period in the same medium with or without added insulin (0.2 unit/ml). At the end of this period the initial velocities of aminoisobutyrate uptake were determined over a 0.1–12.8 mM range of analogue concentrations in experiments of 5 min duration. Uptake values were corrected for the non-saturable component of transport [17, 18] and analysed by graphical transformations [19] and computer methods [6, 9].

Fig. 2 shows the plot v versus $v/[S]$ for aminoisobutyrate uptake (saturable process) by cells incubated with or without added insulin under conditions of inhibited protein synthesis. As expected for an amino acid which is known to enter the cell by

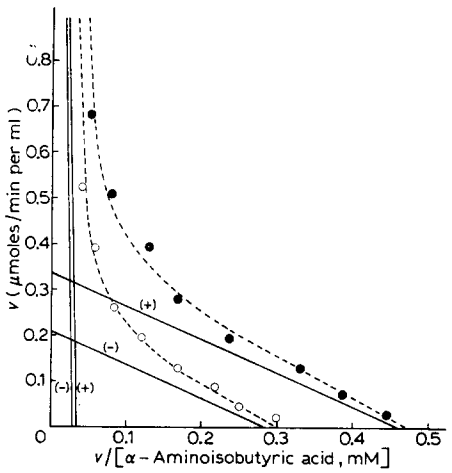

Fig. 2. Initial velocities (v) of α-aminoisobutyric acid uptake (saturable process) by isolated cardiac cells against the ratio of velocity to medium concentration: effect of insulin under conditions of inhibited protein synthesis. The data points are means of 2 experiments in the absence (○) and presence (●) of insulin. Broken lines (total aminoisobutyrate transport) are drawn according to the fit of the data obtained by digital computer methods [9]. Continuous lines represent single transport systems in the absence (−) and presence (+) of insulin as derived from values of V and K_m of two overlapping components separated by computer analysis. Kinetic constants for the low K_m component: (a) in the absence of insulin, K_m 0.74 mM and V 0.21 μmoles/min per ml; (b) in the presence of insulin, K_m 0.74 mM and V 0.34 μmoles/min per ml. Owing to the small number of observations at high substrate concentrations, the precision in estimating the kinetic parameters of the high K_m component is rather poor; with this reservation, insulin did not affect V of this component. For the experimental procedure and conditions see text.

more than one transport system (A and L mediations)* the initial velocities of uptake yielded lines concave upwards for points obtained at high extracellular substrate concentrations [6]. Two overlapping components were then separated by programming a digital computer to read out the total transport velocity from the summation of independent Michaelis–Menten expressions [9]. The A mediation was identified with the component characterized by a low K_m in the absence of insulin (cf. ref. 6); the high K_m component disappeared when the measurements were carried out in the presence of sufficient phenylalanine to prevent aminoisobutyrate uptake by the L system. The incubation in the presence of the hormone did not alter K_m and definitely increased V (by approx. 60 %) of this component. This result indicates that insulin accelerates the maximal velocity of amino acid transport by the A mediation even under conditions of inhibited protein synthesis.

Kinetics of degradation of transport proteins

An estimation of the rate of degradation of protein components of Transport Systems A, ASC, L and Ly^+ (and the effect of insulin thereon) has been obtained by measurements of transport activity as a function of time under conditions of inhibited

* No evidence for heterogeneity in the Na^+-dependent uptake of α-aminoisobutyric acid by isolated rat diaphragm has been detected [16].

protein synthesis. The assumption was made that transport activity is proportional to the amount of transport proteins present in the system at any time (cf. ref. 7).

After appropriate pre-incubation in the presence of cycloheximide at a concentration (2 μg/ml) sufficient to inhibit protein synthesis almost completely [7], cardiac cell suspensions were incubated in the same medium with or without added insulin. Transport activity was determined every 30–45 min (for 150–180 min) by labelled amino acid uptake in 5-min experiments (initial velocity) in the presence of discriminating substrates (cf. ref. 12).

Fig. 3 shows that the activity of the A transport system decreases as a single exponential with a half-life of approx. 190 min when cells are incubated in the absence of insulin; in the presence of the hormone, transport activity decreases more slowly with time (half-life of approx. 600 min). Comparable results have been obtained when cycloheximide concentration was increased to 5 μg/ml or when puromycin (50 μg/ml) was used and protein synthesis was virtually abolished. A preliminary enhancement of transport activity by this system, as obtained by pre-incubating the cells in amino

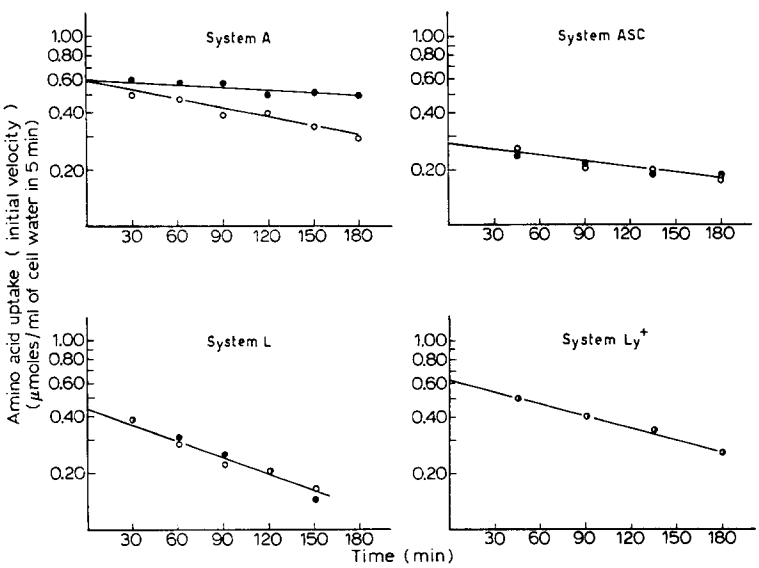

Fig. 3. Changes in amino acid transport activity (Systems A, ASC, L and Ly^+) of cardiac cells incubated under conditions of inhibited protein synthesis: effect of insulin. Cell suspensions, after a 90-min pre-incubation in an amino acid-free Krebs–Ringer buffer (System A) or without pre-incubation (Systems ASC, L and Ly^+), were incubated for 30 min in Krebs–Ringer bicarbonate buffer supplemented with cycloheximide (2 μg/ml) and were then divided into two equal samples. Samples were incubated for an additional 150–180 min in the same cycloheximide-containing medium in the absence (○) or presence (●) of insulin (0.2 unit/ml). Incubations were at 37.5 °C in an atmosphere of O_2–CO_2 (95 : 5, v/v). Amino acid transport activity was measured every 30–45 min on aliquots of the cell suspension (10^7 cells) transferred into flasks containing the same medium supplemented with 0.1 mM labelled amino acid and 5 mM discriminating substrate(s) as specified below, and incubated for 5 min at 37.5 °C. System A: α-amino[^{14}C]isobutyric acid uptake in the presence of L-phenylalanine; System ASC: L-[^{14}C]serine uptake in the presence of MeAIB and L-phenylalanine; System L: L-[^{14}C]leucine uptake in the presence of MeAIB and GPA; system Ly^+: L-[^{14}C]-lysine uptake in the presence of L-phenylalanine. The data are plotted as a function of time; note logarithmic scale of the ordinate.

acid-free medium [6], was required in these experiments to reach initial rates of amino acid uptake which were sufficiently high for correct measurement (cf. refs 7 and 12). As expected, insulin did not affect the rate of change in transport activity for Systems ASC, L and Ly^+. All the results obtained in insulin-free incubations confirm those reported previously [7, 12].

The effect of the hormone is consistent with a protection against inactivation of protein components of Transport System A. A direct action of insulin on transport molecules appears unlikely since the hormone increases transport maximum (V) without changes in K_m even under conditions of inhibited protein synthesis (see the preceding paragraph).

Transcription and translation of mRNA coding for transport proteins

We have reported previously [7] that cardiac cells, incubated in the presence of sufficient cycloheximide to inhibit protein synthesis, retain the capacity to synthesize (and accumulate) mRNA coding for proteins involved in amino acid transport by the A mediation. Since cycloheximide can be easily removed from cells by proper washing procedures and the synthesis of RNA is suppressed by actinomycin D [7], we had the possibility to investigate separately transcription and translation as sites of insulin regulation on the activity of Transport System A.

The adopted procedure consisted of a preliminary incubation of isolated cardiac cells in the presence of cycloheximide (2 μg/ml) with or without added insulin (phase of inhibited translation) followed by an incubation period of inhibited transcription (by actinomycin D) in the absence and presence of insulin. Appropriate washings

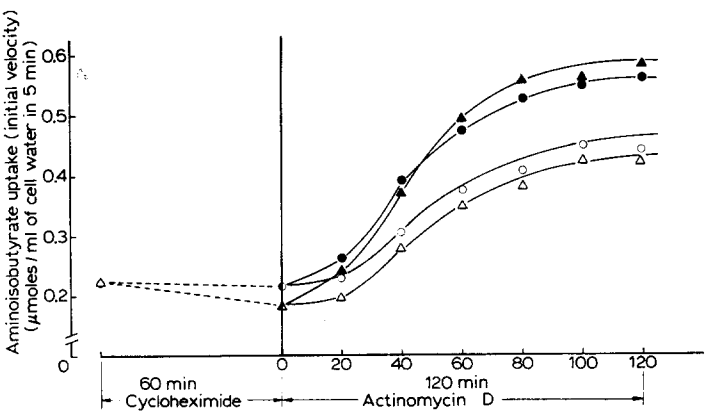

Fig. 4. Changes in amino acid transport activity (A mediation) of cardiac cells incubated in the presence of actinomycin D after pre-incubation in cycloheximide; effects of the addition of insulin to pre-incubation and/or incubation media. Cells, pre-incubated for 60 min in a cycloheximide-containing (2 μg/ml) Krebs–Ringer buffer in the absence (△, ▲) or presence (○, ●) of insulin, were washed, transferred to Krebs–Ringer buffer containing actinomycin D (5 μg/ml) in the absence (△, ○) and presence (▲, ●) of insulin and incubated for an additional 120 min. Pre-incubation and incubation were at 37.5 °C in an atmosphere of O_2–CO_2 (95 : 5, v/v). When present insulin was 0.2 unit/ml. 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.

ensured removal of inhibitor molecules before changes in medium composition and before measurements of amino acid transport by System A. Activity was measured by aminoisobutyrate uptake in experiments of 5 min duration (initial velocity) at various time intervals throughout the incubation.

Fig. 4 shows that isolated cardiac cells, pre-incubated for 60 min in a medium containing cycloheximide, subsequently exhibited a net increase of transport activity when transferred into a medium containing actinomycin D (cf. ref. 7). The addition of insulin to the cycloheximide-containing medium protected transport proteins against inactivation during this phase (cf. Fig. 3) but did not affect the rate of change of transport activity during subsequent incubation in cycloheximide-free actinomycin D-containing medium. However, the addition of insulin during the second phase (inhibited transcription, active translation of pre-accumulated mRNA) definitely enhanced the rate of change in transport activity. No further increment of activity during this phase has been observed when the hormone was present since the beginning of preliminary incubation. 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. 4 indicate that insulin does not affect the rate of mRNA transcription during the phase of inhibited translation and accelerates mRNA translation during the phase of inhibited transcription. Hence, the hormone appears to act at a post-transcriptional site. An insulin-mediated stimulation of protein synthesis at the ribosomal level in muscle has been reported by Stirewalt et al. [20]; the hormone appears to exert a translational control at the level of peptide chain initiation [21–23].

DISCUSSION

The transport of amino acids across the cell membrane in animal tissues is accomplished by a number of systems of mediation whose specific properties include different reactivity to substrates, ion-dependence, sensitivity to pH and metabolic inhibitors, capacity for exchange diffusion and trans-inhibition and occurrence of adaptive regulation [5, 13, 24, 25]. A stimulatory effect of insulin on amino acid uptake has been reported for such tissues as muscle [8, 17, 26, 27], bone [28], lymphoid cells [29], mammary epithelial cells [30] and hepatoma culture cells [31]. In these studies no attempts were made to identify the transport system(s) involved in hormonal regulation. The results presented in this paper indicate that among the systems found operative in chick embryo heart cells for the transport of neutral and basic amino acids, only the A mediation is responsive to insulin. Evidence that insulin acts predominantly or entirely on Transport System A in rat diaphragm has been recently reported by Riggs and McKirahan [16]. The latter agency is also the sole transport system which exhibits adaptive regulation [12]. Both adaptive and insulin regulation affect the availability of specific transport proteins by modulating the rate of their synthesis and degradation. However, they operate at a different site of the synthetic process and display an opposite effect on the inactivation of these protein components. Adaptive control of the A mediation involves a repression-depression mechanism by its substrate molecules acting on the transcription of specific mRNAs coding for transport proteins [7]. Insulin regulation of the synthesis of these molecules

appears to be post-transcriptional. A post-transcriptional control of enzyme induction and repression by steroid hormones has been suggested by Thomkins et al. [32]. Both adaptive and hormonal regulation affect transport protein degradation at the membrane level. However, repressive conditions (presence of pertinent amino acid substrates) enhance the rate of inactivation of transport proteins of the A system (increased degradation during the operation) [7, 12], whereas insulin decreases this rate, apparently protecting protein components against degradation. Relevant to the latter point is the fact that insulin is the principal, and perhaps the sole hormone capable of lowering the rate of muscle proteolysis [33].

An indication that insulin stimulates amino acid transport by skeletal muscle in two ways comes also from the work of Elsas et al. [34] who reported that the sensitivity of α-aminoisobutyric acid uptake by the rat diaphragm to insulin and inhibitors of protein synthesis changes during postnatal development. In younger animals, puromycin inhibited both resting uptake and the stimulatory effect of insulin. In older animals, exposure to the inhibitor did not affect resting transport, but abolished insulin stimulation. These studies support a dual role for the hormonal stimulatory effect (one protein-dependent, and another independent of new protein synthesis). They imply that the resistance to inhibitors of protein synthesis which occurs with increasing age is associated with a progressive decrease in the turnover rate of transport proteins. In this line, our results suggest that: (a) insulin preferentially affects the rate of degradation of rapidly turning-over transport proteins at the cell membrane during early developmental stages (when overall protein synthesis is likely to occur at a close to maximal rate and insulin does not accelerate this process [34, 35]); (b) insulin modulates the rate of synthesis of transport proteins, perhaps enhancing peptide chain initiation [21, 23], during adult life (when the rate of protein synthesis decreases, insulin acceleration of the synthetic process becomes apparent, and the turnover of postulated carrier proteins slows down to negligible values [34]).

Insulin stimulation of amino acid uptake by chick embryo heart is operative at all stages of embryological development [36]. A saturable transport system for glucose, however, appears and becomes sensitive to insulin between the seventh and the ninth day of development [37], coincidental to the appearance of the first granulations (which are believed to be a sign of insulin secretion) in the β-cells of the embryonic pancreas [38]. This discrepancy requires the introduction of an arbitrary distinction between the effects of insulin (or of a postulated mediator formed at the cell membrane following the interaction of insulin with specific receptors [39]) on differentiated intracellular functions. The fact that a pronounced decrement in the concentrative capacity for amino acids by the cardiac cell takes place between the seventh and the tenth day of embryological development [1] indicates, however, that also in this case insulin becomes available (and hormonal stimulation becomes physiologically relevant) when the activity of amino acid transport undergoes a marked transition during ontogeny. It is pertinent that insulin has been identified as one of the growth-promoting and anabolic substances which activate the pleiotypic program of the cell, i.e. a set of metabolically unrelated biochemical reactions (including the overall rate of protein and RNA synthesis. protein degradation, and membrane transport of glucose, amino acids and nucleic acid precursors) capable of fluctuating in coordination when changes in the environment affect cellular growth [40, 41].

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