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AMINO ACID TRANSPORT IN PLACENTAL SLICES

MECHANISM OF INCREASED ACCUMULATION BY PROLONGED INCUBATION

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SUMMARY

The accumulation of α-aminoisobutyric acid by placental slices is increased dramatically upon prior incubation of the slices in amino acid-free, buffered saline. This increase is inhibited by inhibitors of protein synthesis and is accompanied by an increased V for the transport process. While alternative explanations are discussed, these data suggest that the incubation effect may be mediated through an increase in the number of available transport sites which are synthesized during the incubation period. Incubation with an amino acid mixture diminishes the increase as well as general protein synthesis, suggesting that a reduced availability of amino acids may initiate compensatory changes in the synthesis of cellular transport proteins.

INTRODUCTION

Amino acid transport studies using tissue slices are usually preceded by incubation in a buffered medium lacking the radioactively labelled amino acid, so as to achieve a steady state of tissue electrolytes. In studies on the immature rat uterus [1] and chick embryo heart [2–4] it has been observed that the rate of amino acid uptake by tissue slices is proportional to the duration of the preliminary incubation period. Furthermore, kinetic evidence and experiments with inhibitors of protein synthesis suggested that increases in amino acid transport proteins are responsible for the preincubation* effects.

The human term placenta accumulates α-aminoisobutyric acid (aminoisobutyrate) by an energy-dependent process which follows Michaelis-Menten kinetics [5]. In a report of work carried out in another context, we noted that aminoisobutyrate accumulation by human placental slices occurs at a dramatically greater rate following a 3 h preincubation, as compared with the usual preincubation period of 45 min [6]. This preliminary observation in the placenta has been confirmed and stud-

* To minimize confusion, the term preincubation is used to denote the preliminary incubation of tissue in the absence of radioactively labelled amino acid.

ied by Smith and co-workers [7, 8] and the effect has also been observed in newborn rat kidney cortex [9]. The present studies further explore the mechanism of this phenomenon in human placental tissue.

MATERIALS AND METHODS

α-Aminoisobutyric acid accumulation in placental slices was measured as described previously [6]. Essentially, this procedure consisted of incubating fresh human term placental tissue slices in balanced Krebs-Ringer bicarbonate (pH 7.3) for various time periods (the preincubation), then transferring the slices to another flask containing buffered saline plus 0.1 mM aminoiso[^{14}C]butyrate. After incubating the slices in the presence of the labelled amino acid for 1 h at 37 °C (at which point uptake is proceeding linearly), the ratio of intracellular to extracellular aminoisobutyrate was calculated after correcting for the extracellular water space.

For estimating the level of protein synthesis occurring in the slices after various modifications in the preliminary incubation, the incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable material was measured. Slices were incubated with radioactive leucine for 20 min, removed, blotted and homogenized in 10 % trichloroacetic acid. After centrifugation, the trichloroacetic acid-insoluble pellet was washed 3 times, weighed and transferred quantitatively to a scintillation counting vial. To decolorize the tissue, 0.5 ml of H_2O_2 was added and allowed to remain in contact with the tissue for about 1 h. The tissue was then solubilized by the addition of 2 ml of protosol followed by incubation of the vials in a 60 °C water bath until digestion was complete. Then 10 ml of a toluene/PPO/POPOP/Triton X-100 scintillation cocktail was added, and the radioactivity was measured in a liquid scintillation counter after chemiluminescence had subsided.

RESULTS

Fig. 1 shows the linear relationship between duration of preincubation (in the absence of aminoisobutyrate) and extent of amino acid accumulation subsequently obtained after incubation in the presence of aminoiso[^{14}C]butyrate. The accumulation ratio (aminoisobutyrate inside the cells/aminoisobutyrate outside the cells) after either a 1- or a 2-h incubation with aminoisobutyrate increased approximately threefold when tissue slices were preincubated for 3 h as compared to 15 min. The two most obvious possible explanations for the mechanism of this phenomenon are:

(1) Longer preincubation times allowed ongoing synthesis of membrane-associated carrier molecules making more transport sites available.

(2) Existing transport sites were modified resulting in greater transport efficiency.

If only the former possibility were true, kinetic studies should show an increase in the maximum uptake rate, V, for the transport process resulting from the increased number of carrier molecules, with little or no change in the Michaelis constant, K_m. In addition, inhibitors of protein synthesis might interfere with the preincubation effect if the synthesis of new carrier protein was involved. On the other hand, if the effect resulted from increased efficiency of existing transport sites, one might expect a decrease in K_m with no increase in V and a resistance to any inhibitor effect.

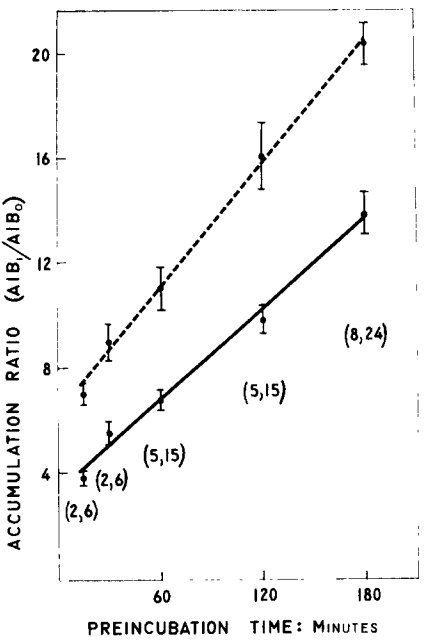

Fig. 1. The effect of preincubation on the rate of α-aminoisobutyrate (AIB) accumulation by placental tissue slices. The slices were preincubated in amino acid-free Krebs-Ringer bicarbonate (pH 7.3) for the times indicated and were then transferred to flasks containing α-aminoiso[^{14}C]butyrate for a 1 h (——) or 2 h (– – –) incubation at 37 °C. The first number in parentheses indicates the number of placentas used and the second number is the total number of measurements. The data points indicate the mean ± S.E.

Fig. 2A shows the results of a kinetic study in which the 1-h accumulation of different concentrations of aminoisobutyrate was measured following different preincubation times. Fig. 2B shows an expanded plot in the vicinity of the origin. The data can be grouped into two sets, each having kinetic similarities. From zero to 45 min preincubation, the data demonstrated no substantial change in the V value, but showed a decrease in K_m. This result suggests that the number of available transport sites did not initially increase, but that the transport efficiency of existing sites increased. This increase might have occurred through some conformational modification which afforded enhanced affinity of the transport machinery for the amino acid. Beyond 45 min preincubation, there was no further change in K_m, but V increased. This increase might have occurred if new transport sites became available, as through the new synthesis of carrier molecules. While these data afford suggestive comparisons, certain reservations are appropriate. In the data set shown in Fig. 2B1, it is not known whether anomalies due to inadequate tissue-buffer equilibration might result from abnormally short preincubation periods.

The effects of inhibitors are shown in Fig. 3. A 2-h preincubation in the presence of cycloheximide, puromycin or actinomycin D depressed the subsequent α-aminoisobutyrate accumulation at 1 h to 41–51 % of control.

In vivo, tissues are exposed to circulating amino acid concentrations of the order of 0.1 mM. It therefore seemed possible that prolonged preincubation in the

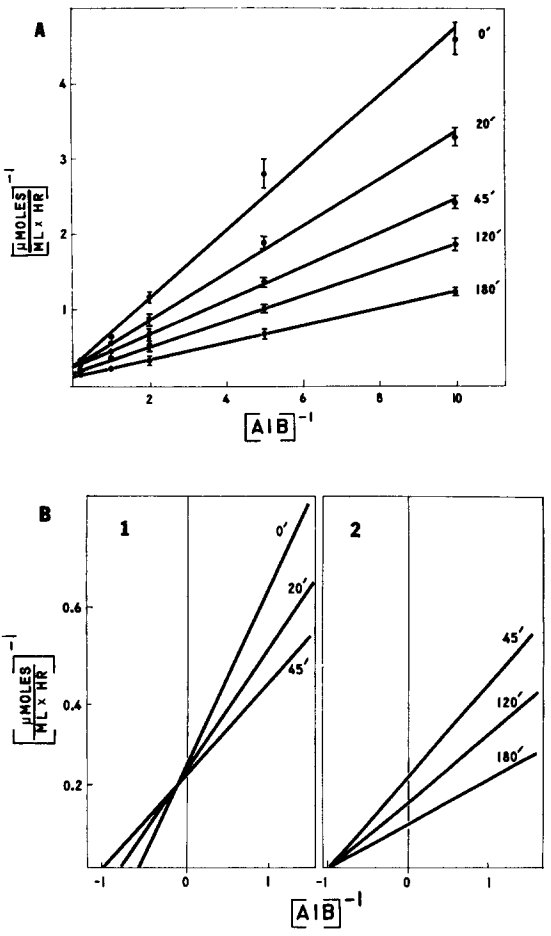

Fig. 2. A. Double reciprocal plot of the accumulation rate as a function of α-aminoisobutyrate (AIB) concentration (mM). The lines were fitted to the points by the method of least squares. The numbers associated with each line represent preincubation times. The incubation with α-aminoiso-[^{14}C]butyrate was for 60 min in each case. The 0 preincubation time points are each derived from 6 measurements on one placenta; those for the 20 and 120 min preincubations represent 6 measurements each on each of 2 placentas; those for the 45 and 180 min preincubations are from 6 measurements each on each of 3 placentas. B. Expanded scale drawing of the data in the vicinity of the origin.

absence of any amino acids might have produced compensatory changes in the cellular machinery involved with amino acid uptake. Fig. 3 also shows that the presence of amino acids reduced the effect of the preliminary incubation.

Since preincubation with inhibitors interfered with the normal increase in α-aminoisobutyrate accumulation but did not abolish it, it was desirable to measure the extent of the effect of inhibitors on protein synthesis in the placental slices. In addition, it was important to know how various preincubation modifications affected the level of protein synthesis, since protein synthetic activity and transport might be tightly coupled processes. Even if new synthesis of transport molecules was not abolished by

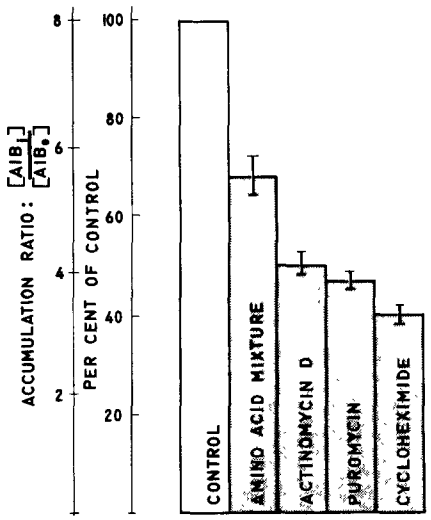

Fig. 3. The influence of inhibitors of protein synthesis and an amino acid mixture on the α-aminoisobutyrate (AIB) accumulation ratios after 1 h. Placental slices were preincubated for 2 h in the presence of cycloheximide (0.1 mM), puromycin (0.1 mM), actinomycin D (1.0 mM), an amino acid mixture (0.1 mM in each of the 19 common amino acids, excluding asparagine and glutamine) or no inhibitor. At the end of the preincubation period, the slices were blotted and transferred to a flask containing 0.1 mM α-aminoiso[^{14}C]butyrate for a 1 h incubation at 37 °C. Each value represents the average of 6 measurements on each of 3 placentas ±S.E.

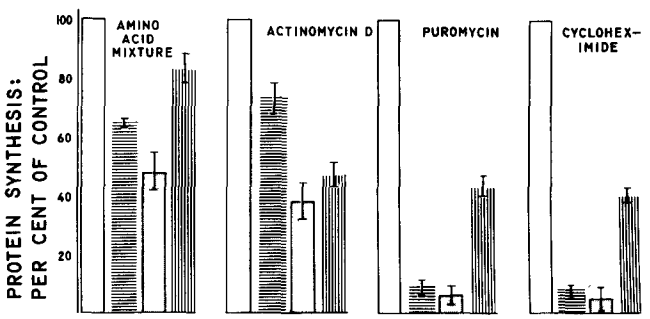

Fig. 4. The effects of inhibitors and an amino acid mixture on protein synthesis in placental tissue slices. Slices were preincubated with the same concentrations of constituents as given in Fig. 3 (except that leucine was omitted from the amino acid mixture so as to avoid diluting the specific activity of the [^{3}H]leucine). They were subsequently incubated with labelled leucine for 20 min. The horizontally striped bars show [^{3}H]leucine incorporation into trichloroacetic acid-precipitable material after a 45 min preincubation in the presence of the indicated constituents. The lightly stippled bar represents a 120 min preincubation with the inhibitors. The vertically striped bar shows the leucine incorporation after a 120 min preincubation followed by a washing of the slices in inhibitor-free medium for 40 min followed by the 20 min incubation with [^{3}H]leucine. The purpose of this was to estimate the extent of recovery of protein synthesis by 1 h after preincubation (since the accumulation studies utilized a 1 h incubation with labelled amino acid). The values for the 45 and 120 min preincubations not followed by washing represent the average of 2 measurements on each of 2 placentas ±S.E. The values for the washed slices are the averages of 2 measurements on each of 6 placentas. Each set is shown with a clear, 100 % control bar for comparison.

the inhibitors, our results might be accounted for by reduced transport accompanying a reduced amino acid requirement, which in turn resulted from reduced protein synthesis. This signal of reduced need could be transmitted to the transport apparatus by some mechanism other than a decreased synthesis of transport molecules.

Fig. 4 shows the effects of inhibitors and the amino acid mixture on protein synthesis. These modifications all reduced protein synthesis and were at least partially reversible. As expected, puromycin and cycloheximide almost abolished protein synthesis during a 45 min preincubation period. Actinomycin D produced some decrease in protein synthesis after 45 min, but showed a considerably greater effect when applied during a 3-h preincubation. This behavior is also reasonable since actinomycin D affects protein synthesis indirectly, having its primary influence on RNA synthesis. Perhaps unexpectedly, preincubation with the amino acid mixture also resulted in a depression of protein synthesis. In each case, the inhibition was partially reversed by washing the slices prior to incubation with the labelled leucine.

DISCUSSION

Christensen and co-workers [10, 11] showed that fetal guinea pig liver accumulates certain amino acids to a much lower extent than maternal tissue. Within 24 h after birth, however, this discrimination largely disappears as the ability of the newborn liver to accumulate glycine, cycloleucine and α-aminoisobutyrate rapidly increases. This rapid postnatal transport of amino acids into guinea pig liver may be initiated by the abrupt fall in plasma amino acids which occurs after delivery [12]. This possibility has received subsequent support from observations of increased amino acid accumulation following preincubation of fetal or immature tissues in amino acid-free media [1–4, 6–9]. The observation of Reynolds et al. [9] that fetal kidney cortex, but not that of the adult, shows the preincubation effect is in accord with this supposition. So is our present finding that preincubation of placental tissue in the presence of amino acids diminishes the increase.

The molecular mechanism by which the rate of accumulation is increased during preincubation of placental slices in an amino acid-free medium is of great interest. Smith et al. [7] reported that prolonged preincubation of placental fragments resulted in an increased V and a decreased K_m of the transport process. Our data suggest that the reduction in K_m occurs early in the preincubation period, and that later increases in accumulation rate result primarily from an increased V. This increase in V suggests that the number of available transport sites in placental cells may be increasing secondary to the synthesis and deposition into the membrane of new carrier molecules. This notion is supported by our present data and those of Smith and Depper [8], which show that inhibitors of protein synthesis substantially decrease the preincubation rise in the rate of aminoisobutyrate accumulation.

There are at least two possible explanations for the effect of inhibitors. One is the possibility that the synthesis of transport molecules is inhibited. The other is that a general inhibition of protein synthesis (even in the absence of transport protein synthesis) could result in decreased amino acid requirement. This in turn could further result in a reduced uptake rate through some tight coupling mechanism with the transport machinery. Our evidence is most consistent with the former possibility. Treatment with puromycin and cycloheximide reduced protein synthesis to less than 10 %

of control. This identical treatment only prevented the preincubation-induced increase in accumulation ratio, but did not reduce the ratio below that observed with no preincubation (compare Fig. 3 with Fig. 1). If tight coupling between protein synthesis and transport took place, inhibition of protein synthesis to less than 10 % of control should have caused a similar diminution in the accumulation ratio. In other words one might envision the fresh tissue as possessing a certain number of transport sites. This number increases following preincubation in the absence of amino acids. Partial inhibition of protein synthesis (as with actinomycin D or by an amino acid mixture) may reduce the formation of additional sites, but even complete inhibition does not reduce the level below that number present initially.

The cause of the reduction in the preincubation-mediated increase when preincubation is carried out in the presence of an amino acid mixture is unclear. Amino acids may reduce accumulation by an effect on general protein synthesis, rather than by a selective influence on the transport machinery.

A cautionary comment seems in order. While we and the others reporting this phenomenon have emphasized a mechanism involving an altered rate of transport into cells, it is also possible that an altered rate of amino acid exodus from the cells may be at least a contributory influence. For example, Webber showed that slices of newborn rat kidney cortex concentrated aminoisobutyrate to a greater extent than adult cortex because of a slower rate of aminoisobutyrate exodus in the young tissue [13, 14]. Also, while a preliminary incubation of placental slices with alanine decreases the preincubation effect [8], this treatment also increases the rate of aminoisobutyrate exodus from the tissue [5].

This system may afford a method of manipulating the synthesis of membrane-associated proteins which could be a considerable asset in studying the nature of production, translocation and intramembrane deposition of a specific protein. It also suggests the possibility that the placenta utilizes its ability to increase the accumulation of amino acids during periods of maternal starvation so as to spare the fetus from amino acid deprivation.

REFERENCES

1 Riggs, T. R. and Pan, M. W. (1972) Biochem. J. 128, 19
2 Gazzola, G. C., Franchi, R., Saibene, V., Ronchi, P. and Guidotti, G. C. (1972) Biochim. Biophys. Acta 266, 407
3 Franchi-Gazzola, R., Gazzola, G. C., Ronchi, P., Saibene, V. and Guidotti, G. C. (1973) Biochim. Biophys. Acta 291, 545
4 Gazzola, G. C., Franchi-Gazzola, R., Ronchi, R. and Guidotti, G. C. (1973) Biochim. Biophys. Acta 311, 292
5 Miller, R. K. and Berndt, W. O. (1974) Am. J. Physiol. 227, 1236
6 Longo, L. D., Yuen, P. and Gusseck, D. J. (1973) Nature 243, 531
7 Smith, C. H., Adcock III, E. W., Teasdale, F., Meschia, G. and Battaglia, F. C. (1973) Am. J. Physiol. 224, 558
8 Smith, C. H. and Depper, R. (1974) Ped. Res. 8, 697
9 Reynolds, R., Rea, C. and Segal, S. (1974) Science 184, 68
10 Feldman, B. and Christensen, H. N. (1962) Proc. Soc. Exptl. Biol. Med. 109, 700
11 Christensen, H. N. and Clifford, J. B. (1963) J. Biol. Chem. 238, 1743
12 Christensen, H. N. (1973) Fed. Proc. 32, 19
13 Webber, W. A. (1968) Can. J. Physiol. Pharmacol. 46, 165
14 Webber, W. A. (1968) Can. J. Physiol. Pharmacol. 46, 765