Biochimica et Biophysica Acta, 345 (1974) 17–26

BBA 76605

TRANSPORT OF AMINO ACIDS IN RAT PANCREAS DURING DEVELOPMENT

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(Received November 5th, 1973)

SUMMARY

1. The transport of α-aminoisobutyric acid, α-aminocyclopentanecarboxylic acid and glycine was studied in vitro in relation to the development of the rat pancreas.

2. At all ages (fetal and neonatal), the pancreas is able to accumulate these amino acids to a level in excess of the medium concentration.

3. The highest accumulation is observed at the earliest fetal period examined (4 days prior to birth). Transport falls at birth to a minimal level then increases to a maximum at 2 days of age.

4. The relatively low level of accumulation seen at birth is not due to a change in the rate of exodus of amino acids which is comparable at all ages ($K_d = 0.023\ min^{-1}$).

5. The transport system is sensitive to extracellular Na^+ before and 24 h after birth but is little affected by Na^+ within the first hours after birth.

6. The transport system is nearly completely inactive at 2 °C. Replacement of oxygen by nitrogen does not affect the transport activity in the fetal or neonatal pancreas. Dependence of transport on O_2 increases progressively with the age of the animal.

7. Lowered ATP levels cannot account for the drop in transport activity at birth.

INTRODUCTION

In recent years, interest has grown in the development of transport mechanisms for substances of biological importance, such as sugars and amino acids. A more thorough understanding of these problems might reveal a possible relationship between events at the plasma membrane surface and events inside the cell and perhaps provide evidence pertaining to the mechanism of transport. Christensen and Clifford [1] were amongst the earliest investigators to examine the ontogeny of amino acid transport in the liver of the fetal and neonatal rat. They showed that liver does not accumulate amino acids at the fetal stage and that true transport systems develop after birth. It was reported that certain enzymes characteristic of the liver were almost completely absent before birth (Greengard [2]). There has been no suggestion of a causal relationship between the almost simultaneous appearance of these enzymes and the transport mechanism for amino acids.

Studies by Baerlocher et al. [3, 4] and by Segal et al. [5] have shown that the development of amino acid transport systems in kidney is a complex process, different for each amino acid tested. Glycine and proline, each are transported by two mechanisms, one with a low K_m and high capacity, the other a high K_m and low capacity. The temporal development of these transport systems is different and the pattern of amino acid exodus is different at birth from that seen in an adult organ, thus further increasing the difficulty in interpreting the data.

In contrast to liver and kidney, the transport of amino acids in the small intestine is at a maximum at birth or within a few hours of birth and declines with increasing age of the animal (Fitzgerald et al. [6]). In this tissue, Pratt and Terner [7] have shown that the pattern of development of an active transport mechanism is variable depending on the particular amino acid in question. Thus, transport activity for lysine appears four days before birth, valine, two days prior to birth and glycine transport on the day following birth. In fertilized sea urchin eggs, Epel [8] showed that there is a relationship between the appearance of a protein-synthesising mechanism and an active, Na^+-dependent transport system. It appears that the development of this type of transport is coupled to the formation of a K^+-sensitive membrane potential.

The appearance of a Na^+-dependent transport system was also shown in the lymphocyte whose growth and differentiation were stimulated by Concanavalin A. (van den Berg et Betel [9, 10]).

Although the adult rodent pancreas is extremely active in amino acid transport and accumulation, no studies have appeared on the temporal aspects of development of its transport systems for amino acids. The exocrine pancreas is a tissue whose cytodifferentiation is well documented (Perrier [11]; Pictet et al. [12]; Przybylski [13–15]; Sjostrand [16]; Snook [17]; Wessel and Evans [18]; Rutter et al. [19]; Kemp et al. [20]). It is also an organ whose full function is called into full play after birth, since it might be anticipated that the secretion of digestive enzymes is not required in the fetal state. Therefore it occurred to us that if the high amino acid transport activity were in some manner associated with the high rates of secretory protein synthesis in this organ, there should be dramatic changes in amino acid transport as a function of age of the animal.

Our first objective was to characterize an amino acid transport system at the earliest times possible and to examine its activity and characteristics in relation to age of animal.

MATERIALS AND METHODS

Sprague–Dawley rats were used in these experiments. To determine the age of the fetus, two methods were used; (a) days after the appearance of a vaginal plug and (b) the mean length of the fetus according to Altman and Dittmer [21]. In general, age determination by the two methods was in close agreement. To obtain the fetus prior to the normal end of the gestational period, the dam was anaesthetized with Nembutal (40 mg/kg body wt) and the young delivered by Caesarian section. The fetuses were cooled, decapitated and the pancreas excised rapidly. Animals of other ages were also decapitated prior to excision of the pancreas.

Before incubation, the pancreas was trimmed of adhering connective tissue and preincubated in oxygenated Krebs–Ringer phosphate buffer. The duration of the preincubation did not exceed 15 min. The composition of the standard incubation medium was: NaCl, 145 mM; KCl, 5.8 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.45 mM; KH_2PO_4, 0.2 mM; Na_2HPO_4, 2 mM at pH 7.4. Where indicated NaCl was replaced by an equivalent concentration of choline chloride. The medium was oxygenated for about 10 min prior to use.

Pancreas from animals up to 10 days post partum was incubated intact. Pancreas from animals 10 days and over was cut into sections of about 5 mg.

Measurement of amino acid uptake

The pancreas was incubated in a shaking water bath in Erlenmeyer flasks containing buffered Krebs–Ringer and the ^{14}C-labelled amino acid. Gassing with O_2 (or N_2) continued for the duration of the experiment.

After incubation, the pancreas was removed and centrifuged for 30 s at 800 × *g* in a tared tube. The supernatant fluid was removed, the inside of the tube wiped dry and the tube plus contents reweighed. This weight represents fresh weight. Then the pancreas was homogenized with 1.0 ml of 5% trichloracetic acid in a homogenizer with a Teflon pestle (Arthur H. Thomas, Philadelphia, Pa.), centrifuged and an aliquot of the supernatant counted in a liquid scintillation counter, using a modified Bray's scintillation solvent [22]. When glycine was used as the ^{14}C-labelled amino acid, the precipitate was washed twice with 5-ml aliquots of 5% trichloracetic acid, dissolved in 0.2 ml 2 M NaOH and counted by liquid scintillation. To measure exodus of amino acids, the pancreas was incubated for 30 min with the ^{14}C-labelled amino acid, and then transferred to another flask containing the same incubation medium but devoid of the amino acid in question and incubated for 10–20 s. The pancreas was transferred to a second flask containing amino acid-free, fresh incubation medium and incubated for 5 min. This process was repeated for a period of 45 min at 5-min intervals (9 transfers).

Extracellular space was measured simultaneously with [^3H]dextran or with separate samples using [^{14}C]dextran (60 000–90 000 mol. wt) or inulin (5000–6000 mol. wt). The experimental procedure was identical to that for uptake. To determine extracellular space, the following computation was used.

$$\text{extracellular space} = \frac{\text{cpm/mg fresh weight tissue}}{\text{cpm/}\mu\text{l medium}} = \mu\text{l/mg fresh weight}$$

Dry weights of the tissue were obtained by drying the tubes containing tissue pellets to a constant weight at 150 °C. The difference, fresh weight–(dry weight+extracellular space) is equal to intracellular weight. The relationship between dry weight, wet weight and intracellular space is given in Table I.

Determination of $Na^+ + K^+$

Tissues were dissolved in concentrated HNO_3, and diluted to known volume with glass-distilled water and assayed by flame photometry.

Estimation of α-amylase

α-Amylase was determined by the method of Bernfeld [23] using a 2% solution of soluble starch and 1 min for reaction time instead of 3 min as in the original procedure.

TABLE I

DRY WEIGHT, EXTRACELLULAR AND INTRACELLULAR VOLUMES OF PANCREAS

Values given are the means±S.D. and are given as percentages of fresh weight. Numbers of experimental observations are in brackets.

Age		Dry weight	Dextran space	Intracellular space
Fetus	18–19 days	12.0±1.7 (8)	29.4±4.9 (8)	58.6
	20–21 days	16.4±0.85 (3)	30.3±5.5 (7)	53.3
Newborn	at birth	21.9±3.7 (30)	30.4±2.6 (7)	47.7
	2 days after birth	12.0±1.6 (31)	41.0±4.3 (27)	47.0
Adult		23.0±2.1 (18)	33.4±7.2 (7)	43.6

Estimation of ATP

ATP was determined by the method of Stanley and Williams [24] using a scintillation counter (out of coincidence) with a gain of 100%.

Immediately after incubation, cold trichloroacetic acid (0.2 ml of 5%) was added and the tissue was homogenized.

Protein content was determined by the method of Lowry et al. [25] using bovine serum albumin as standard.

All radioisotopes used were obtained from New England Nuclear, Boston, Mass. [1-^{14}C]Glycine; α-amino[1-^{14}C]isobutyric acid and α-amino[1-^{14}C]cyclopentanecarboxylic acid (cycloleucine), ^{14}C-carboxy inulin, [^{3}H]dextran and ^{14}C-carboxy dextran were used. All other chemicals were of reagent grade.

RESULTS

The data in Table II show the accumulation at steady state of three amino acids as a function of age. A similar qualitative pattern is observed in all cases. The highest accumulation is observed at the earliest fetal period examined. (We found it im-

TABLE II

UPTAKE OF DIFFERENT AMINO ACIDS AT STEADY STATE

The uptake of amino acids in Krebs–Ringer phosphate was examined at steady state (90 min incubation) at 37 °C. Values given are corrected for extracellular (dextran) space. The concentration of the amino acids used was 0.25 mM at a specific activity of 0.1 μCi/μmole.The values are means±S.D. and the number of determinations is in brackets.

Age		Ratio of $\frac{\text{intracellular}}{\text{extracellular}}$ amino acid concentrations		
		α-Aminoisobutyric acid	Cycloleucine	Glycine
Fetus	18–19 days	15.95±2.2 (7)	14.01 (2)	19.02 (2)
	20 days	9.53±0.76 (6)	9.75±1.13 (3)	10.32±0.36 (4)
	21–22 days	8.72±0.43 (8)	10.31±0.58 (3)	7.5 ±0.29 (3)
Newborn	at birth	3.13±0.43 (25)	5.68±1.0 (5)	5.84±0.11 (3)
	2 days, after birth	11.64±1.88 (13)	11.52±1.97 (9)	14.99±1.33 (3)
Adult		8.88±1.36 (20)	9.63 (2)	12.5 ±0.9 (5)

possible to use fetuses younger than 18 days because of fragility and inability to discern the tissue.) It is evident that at all ages the pancreas is able to accumulate these amino acids to a level in excess of the medium concentration, suggesting a process of active transport. The ability to accumulate amino acids appears to fall to a minimal level at birth and then shows a marked increase by two days of age. The most dramatic change occurs within 24 h after birth. The accumulation by the adult pancreas is less than that at two days but not markedly so. A more detailed pattern of α-aminoisobutyrate uptake as a function of age is given in Fig. 1, the inset showing the rate of uptake in newborn and adult rat pancreas.

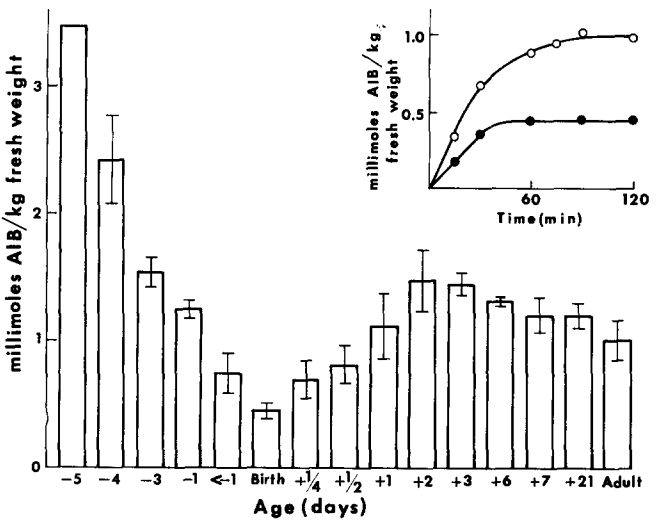

Fig. 1. Uptake of α-aminoisobutyrate into rat pancreas as a function of age of the animal. Conditions of incubation as in Table II.

Since a decrease in accumulation at steady state could be due to a decreased influx or an increased efflux, the variation in exodus with age was determined. Efflux follows first order kinetics with a K_D of 0.023 min^{-1} for adult and fetal and newborn animals. It should be noted that under our experimental conditions there is little likelihood (if any) for recapture of ^{14}C-labelled amino acids since (1) the tissue is transferred to fresh medium every 2–5 min, (2) the cellular α-aminoisobutyrate concentration is always 100 times or more the medium concentration and (3) no more than 70% of the total initial ^{14}C-labelled amino acid is lost in the experimental period.

Na^+ requirement

It has been established that many active transport systems require Na^+ in the incubation medium (for review see ref. 26). We have examined the Na^+-dependence of amino acid transport at different ages of the animal. The results in Table III show that response to Na^+ varies with age. In the newborn, little decrease of uptake is observed upon nearly complete removal of Na^+. In contrast, with pancreas from fetal animals or animals two days and older, a marked drop in accumulation is observed, falling by 30–50% of control values in absence of Na^+. With Na^+_0 at 75 mM

TABLE III

EXTERNAL SODIUM EFFECT ON α-AMINOISOBUTYRIC ACID UPTAKE AT STEADY STATE

Uptakes at steady state (90 min incubation) were determined in normal medium and the media cited below at each of the various ages. The data given are expressed as a percent (±S.D.) of the corresponding control value in normal medium (i.e. 145 mM Na^+). The control values for each of the various ages, expressed in mmoles/kg intracellular water are shown in Table IV, except for fetus 18–19 days where the control value is 3.99±0.55 (7). The values in brackets are the numbers of experimental observations. Experimental conditions as in Table II.

Age		Uptake % of control value		
		$[Na]_o$ = 4 mM	75 mM	145 mM + ouabain 1 mM
Fetus	18–19 days	50.5±10.0 (4)	79.9 (2)	51.0 (2)
	20–21 days	31.3±3.5 (8)	85.3±4.1 (4)	31.0±2.1 (4)
Newborn	at birth	91.0±2.6 (7)	100±3.8 (3)	64.1±7.7 (10)
	2 days after birth	36.8±4.8 (18)	70.8±6.5 (5)	26.5±2.1 (6)
Adult		45.5±10.8 (7)	62.2±9.0 (5)	17.1±0.5 (3)

the inhibition of accumulation is less but still shows the same general characteristics as in 4 mM Na^+. It should be noted that in the near absence of Na^+ (< 4 mM Na^+) the α-aminoisobutyrate concentration in the pancreas at steady state of late fetal and older animals is still at least three times that of the incubation medium and that these values are similar to those obtained in the pancreas of the newborn under optimal conditions (Table II). The presence of ouabain, which has been reported to inhibit amino acid transport [27] in pancreas, results in a greater inhibition of uptake than removal of Na^+, but the relative effects remain about the same, adult pancreas being the most sensitive to ouabain inhibition, followed by 2-day-old and 18–22-day fetal pancreas. It is not clear why ouabain should have a more pronounced effect than Na^+ removal.

The lack of, or the modest effect of, sodium removal in pancreas from fetal animals raises the possibility that some accumulation of α-aminoisobutyrate was not dependent on metabolic activity. For this reason we examined α-aminoisobutyrate accumulation at 2 °C in an oxygen medium and under N_2 (Table IV). It is apparent that at 2 °C there is a very marked decrease of α-aminoisobutyric acid accumulation. With the exception of the adult tissue at 2 °C, the intracellular concentration of α-aminoisobutyric acid does not reach the level of the incubation medium after 90 min incubation. Moreover, little change is observed after 15 min of incubation. With the adult rat pancreas at 2 °C, α-aminoisobutyrate concentration in the tissue becomes equivalent to that of the incubation medium well within 15 min and does not change upon continued incubation for 90 min.

A somewhat different picture obtains under nitrogen. Accumulation by the adult organ is severely but not completely suppressed. In the newborn animal there is no difference between incubation under N_2 and incubation in oxygen, but the uptake in N_2 may be suppressed by iodoacetate. Similar results are obtained with the fetal tissue. However, a few days after birth, accumulation is dependent on the presence of oxygen and in the pancreas from a 2-day-old animal the accumulation in nitrogen is about 50% of that seen in oxygen. These data are consistent with

TABLE IV

α-AMINOISOBUTYRIC ACID UPTAKE AND ATP CONTENT IN DEVELOPING RAT PANCREAS

The pancreas was incubated for 90 min at 37 °C in O_2 or N_2 or at 2 °C in O_2 in Krebs–Ringer phosphate, pH 7.4 or a low sodium medium where choline chloride was used as replacement for NaCl. The values for uptake have been corrected for extracellular space and dry weight and expressed in mmoles/kg cell water. α-Amino[1-^{14}C]isobutyric acid was used at a concentration of 0.25 mM and specific activity of 0.1 Ci/mole. The ATP content was estimated after an incubation period of 60 min in the given medium, and expressed as mmoles/kg fresh weight. The values given are means±S.D. with the number of experimental observations in brackets.

Age	Medium	Experimental conditions				
		O_2		N_2		O_2 (2 °C)
		α-Aminoisobutyrate	ATP	α-Aminoisobutyrate	ATP	α-Aminoisobutyrate
Fetus-2D	Normal	2.18±0.13 (9)	1.59±0.23 (4)	2.24±0.07 (3)	–	0.13±0.02 (3)
Newborn	Normal	0.78±0.11 (25)	0.47±0.09 (9)	0.69±0.07 (5)	0.09±0.02 (5)	0.15±0.04 (5)
Newborn	Low-Na^+(<4 mM)	0.70±0.18 (7)	–	–	–	
2 days old	Normal	2.91±0.47 (13)	1.54±0.1 (7)	1.45±0.15 (3)	0.12±0.02 (4)	0.08±0.02 (14)
2 days old	Low-Na^+(<4 mM)	1.07±0.14 (18)	1.04±0.14 (4)	0.76±0.14 (4)	0.13±0.03 (4)	–
Adult	Normal	2.22±0.34 (20)	1.54±0.14 (3)	0.60±0.16 (5)	–	0.25±0.05 (5)

the known facts that fetal tissue is generally more dependent on glycolytic activity than are adult mammalian tissues and that metabolic activity can be sustained under nitrogen in the fetal animal but not in most adult mammalian tissues. That α-aminoisobutyrate transport in the newborn is still dependent on metabolic activity is suggested by the greatly reduced uptake when temperature is lowered.

In view of our earlier observations [27] that transport activity varies with the ATP level, the possibility was considered that the nadir in transport activity at birth is due to a low ATP level, perhaps due to insufficient substrate availability. However, analysis of the cellular ATP levels as a function of age did not indicate a sufficiently large decrease in ATP level at birth to account for the decrease in the activity although it could contribute to it. As seen in Table IV the ATP level at birth is much lower after incubation under N_2 than under O_2, but there is little difference in α-aminoisobutyrate uptake under the two conditions.

DISCUSSION

The present data show that there are marked changes in amino acid transport activity in the acinar pancreas during the course of fetal growth and in the neonate. Although at the earliest stages of development (4 days prior to birth) the transport activity is high and Na^+-dependent, the activity decreases with age of the animal and reaches a minimal level at birth. It is perhaps significant that the transport activity at birth is not affected by extracellular Na^+ and that when Na^+ is removed from the medium at the other stages of development, the residual transport activity approaches that of the newborn. These data appear to suggest that the Na^+-coupling mechanism decreases in the later fetal stages, goes through a minimum and then increases again. There is no evidence that different transport systems are involved in fetal and adult tissues since kinetic studies of amino acid transport and the effects of other amino acids on α-aminoisobutyrate uptake, suggest that the kinetic parameters are the same in adult and fetal tissues (unpublished observations).

The decreased ATP level at birth is unlikely to be the sole reason for the diminished uptake. If the drop in ATP level from approximately 1.6–0.5 mmoles/kg wet wt accounted for the decrease in uptake at birth, it would be expected that a further drop to 0.1 mM under anaerobic conditions would have a proportionate effect. The data however show that there is little difference in α-aminoisobutyrate uptake in O_2 and N_2 at birth.

The significance of the changing pattern of amino acid transport is unknown. However, certain possibilities are worthy of mention. It is well established that the zymogen granule and amylase content of the pancreas reach nearly maximum levels about four days before birth in the rodent [20]. We have found that within a few hours of birth the level of amylase in the pancreas is sharply decreased, so much so that only 10–20% of the newborn level remains (Table V). Subsequently, the amylase level in the pancreas increases gradually and reaches near adult values after two or three weeks of age (Lin and Johnstone, unpublished). It is during the first two days and more particularly during the first 24 h that transport activity increases.

It is conceivable that during the early stages of fetal development when synthetic activity is high, the transport systems are highly active to provide the tissue with adequate amino acid precursors. However, once the storage of the secretory

TABLE V

CONTENT OF PROTEIN AND α-AMYLASE IN RAT PANCREAS

Protein and α-amylase assays were conducted upon excision of the pancreas from the animals. One unit of amylase is defined as 1 mg maltose liberated in 1 min at 20 °C.

Age		Protein (mg/g fresh weight)	α-Amylase (units/mg protein)
Fetus	2D	151.5± 6.2 (3)	
Newborn	at birth	224.2±25.2 (6)	44.4±8.6 (12)
	2 days after birth	81.1±10.1 (8)	8.5±0.8 (5)
Adult		186.4±20.3 (6)	18.2±3.7 (6)

enzymes is complete and presumably the synthetic systems fully functional, the Na^+-coupling mechanism may be dissociated from the transport system resulting in a decreased transport activity, in line with the decreased demands for protein synthesis. A minimal activity is reached at birth at which time (or shortly after) the stored hydrolytic proteins are released. Subsequently, there is a very rapid restoration of transport function, followed some days later with increased amylase synthesis as measured by the amylase content in the tissue.

No doubt other interpretations are also possible and we are currently examining whether secretagogues and hormones known to increase pancreatic activity may influence the transport of amino acids in the newborn animal.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Medical Research Council and a Fellowship from the Canadian Cystic Fibrosis Foundation to one of us (J.P.C.) 1973, and Fonds National Suisse de la Recherche (1971-72).

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