Biochimica et Biophysica Acta 861 (1986) 389–394
Elsevier

BBA 70345

BBA Report

Transport characteristics of system A in the rat exocrine pancreatic epithelium analyzed using the specific non-metabolized amino acid analogue α-methylaminoisobutyric acid

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(Received 6 June 1986)

Key words: Amino acid transport; Transport system A; Na^+ dependence; Kinetics; α-Methylaminoisobutyric acid; (Rat pancreas)

The selectivity and kinetics of system A amino acid transport in the rat exocrine pancreatic epithelium were characterized using the specific analogue α-methylaminoisobutyric acid. Unidirectional influx of α-methylaminoisobutyric acid was measured in isolated perfused pancreata by rapid dual tracer dilution. In cross-inhibition experiments DL-methylalanine, L-serine, L-cysteine, glycine, L-phenylalanine and L-glutamine were effective inhibitors of influx, whereas L-glutamate and L-lysine were less effective. In the presence of sodium α-methylaminoisobutyric acid influx was saturable with an apparent $K_t = 1.7 \pm 0.2$ mM and $V_{max} = 0.49 \pm 0.03$ μmol/min per g (mean ± S.E., $n = 6$). Influx of α-methylaminoisobutyric acid at 50 μM and 100 μM concentrations was significantly inhibited as the perfusate sodium concentration was gradually decreased from 156 mM to 26 mM by isoosmolar choline replacement. Estimated K_t values for sodium at these two methylaminoisobutyric acid concentrations approximated 200 mM. System A activity in the basolateral membrane of the exocrine pancreatic epithelium exhibits a high transport affinity, a wide tolerance for different amino acids and a dependency upon the extracellular sodium concentration.

Uptake of extracellular amino acids at the basolateral border of the exocrine pancreatic epithelium is mediated by numerous parallel transport systems [1–3] which provide the necessary substrates for the high rate of protein synthesis in this secretory tissue (for review, see Ref. 4). There is growing evidence that the distribution of endocrine tissue throughout the exocrine gland is of physiological relevance [5] and the portal vascular arrangement [6] may convey blood containing high concentrations of islet hormones directly to the basolateral membrane of the exocrine epithelium. Exogenous insulin stimulates the transport activity of small neutral amino acids in the perfused rat exocrine pancreas [7], and in other tissues the classical Na^+-dependent transport system A [8] is believed to be sensitive to hormonal control (for reviews, see Refs. 9 and 10). The interactions between hormone-receptor binding, sodium and amino acid transport protein activation remain to be fully elucidated. System A is unique amongst the amino acid transport systems for its acceptance of *N*-methylated amino acid side chains, and the non-metabolized analogue α-methylaminoisobutyric acid is regarded as a specific substrate for this membrane carrier [11]. In the present study we have used this probe analogue to characterize the specificity, sodium-dependence and kinetic properties of system A in the exocrine epithelium of the perfused rat pancreas.

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Male Sprague-Dawley rats weighing between 150 and 280 g were fasted for 24 h before an experiment but allowed water ad libitum. Animals were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg Sagatal), and the pancreas was surgically isolated from the stomach, spleen and small intestine except for a small duodenal segment attached to the head of the pancreas [2]. The pancreas was perfused at constant flow (1.75 ml/min) via the superior mesenteric and coeliac arteries with an oxygenated Krebs-Henseleit bicarbonate medium containing 5% Dextran T70 (Pharmacia) and 0.25% bovine serum albumin [12]. The isolated preparation was placed in a small temperature regulated bath (mounted in perfusion cabinet at 37°C) containing colloid-free perfusate. The pancreatic venous effluent from the portal vein was diverted past a photoelectric drop recorder to monitor the perfusion rate and then was collected sequentially or passed to waste.

Unidirectional uptake of [^{14}C]methylaminoisobutyric acid (48.4 mCi/mmol, New England Nuclear Chemicals, F.R.G.) by the exocrine pancreatic epithelium was quantified relative to an extracellular tracer D-[^{3}H]mannitol (27 Ci/mmol) using a high resolution dual tracer dilution technique [2,3,13]. Briefly, a bolus injection (100 μl in 1–2 s) of both tracers into the arterial perfusate supplying the pancreas was followed by rapid sequential sampling of the portal vein effluent (30 samples in 60–90 s). Following the sequential venous collection a final 4-min volume was accumulated to maximize tracer recoveries and to quantify tracer amino acid efflux [2,3]. Fig 1B illustrates paired venous tracer dilution profiles obtained for [^{14}C]methylaminoisobutyric acid (MeAIB) and D-[^{3}H]mannitol in an isolated pancreas perfused in the presence of sodium. Amino acid uptake was quantified in each successive venous sample from: uptake = 1 − ([^{14}C]MeAIB/D-[^{3}H]mannitol). The time-course of [^{14}C]methylaminoisobutyric acid uptake is depicted in Fig. 1C, and a maximum uptake of 43 ± 1% was measured 30 s after the injection of isotopes. Uptake remained constant throughout the remainder of tracer dilution experiment but in most experiments a small efflux of [^{14}C]methylaminoisobutyric acid from the epithelium was detected, as

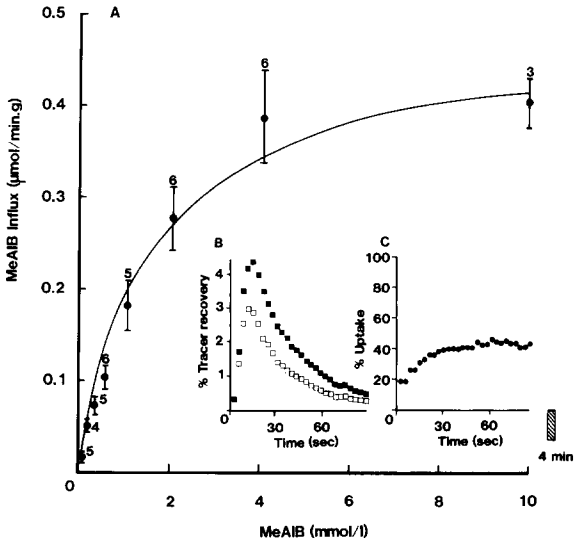

Fig. 1. Kinetics of α-methylaminoisobutyric acid (MeAIB) transport at the basolateral plasma membrane of the exocrine pancreatic epithelium. (A) Michaelis-Menten saturation kinetics for unidirectional MeAIB influx in isolated pancreata perfused successively with up to 8 different concentrations of unlabelled MeAIB (0.05–10 mM). The solid line represents a single rectangular hyperbola fitted to the mean influx values weighted for 1/S.E and the vertical bars denote the S.E. of each mean of n observations in six perfused pancreata. (B) Typical portal vein concentration-time profiles obtained for [^{14}C]MeAIB (□———□) and the extracellular tracer D-[^{3}H]mannitol (■———■) following a bolus injection (100 μl in 1–2 s) of the two tracers into the arterial circuit of an isolated rat pancreas perfused at 1.75 ml/min. Tracer recoveries in the venous effluent were normalized with respect to the injected doses and maximal venous recoveries were observed approximately 15 s after the intra-arterial injection. In this experiment the total recovery of the extracellular tracer D-[^{3}H]mannitol was 95%. (C) The time-course of [^{14}C]MeAIB uptake relative to D-[^{3}H]mannitol in successive venous samples was determined from the two dilution curves shown in Fig. 1B: uptake % = $(1-([^{14}\text{C}]\text{MeAIB}/\text{D-}[^{3}\text{H}]\text{mannitol}))\times 100$. Maximum [^{14}C]MeAIB uptake occurred within 30 s and remained constant for a further 60 s. Following rapid sequential sampling of the venous effluent a single 4-min sample was collected (data not shown in Fig. 1B) to assess tracer efflux using the expression: efflux % = $(1-(U_T/U_{max}))\times 100$ where U_T is the overall uptake of [^{14}C]MeAIB in a 5–6-min period and U_{max} is the maximal fractional uptake for [^{14}C]MeAIB in the rapid dilution experiment (see Refs. 2 and 3). The hatched 4-min column represents the greater venous recovery of [^{14}C]MeAIB relative to D-[^{3}H]mannitol measured in the final accumulated venous sample and indicates a delayed efflux from the pancreatic epithelium.

indicated by the increased venous recovery relative to D-[^{3}H]mannitol in the 4-min sample. The time-course and maximal uptake of methyl-

aminoisobutyric acid in the present in vitro experiments was very similar to that observed previously in pancreata perfused in situ without Dextran T70 but 1% bovine albumin (see Table I, Ref. 2).

Tracer methylaminoisobutyric acid uptake was inhibited by inclusion of unlabelled methylaminoisobutyric acid in the perfusate (data not shown). The kinetics of unidirectional influx were quantified over a wide range of perfusate concentration of unlabelled substrate (0.05–10 mM) using the expression: influx $= -F \cdot \ln(1 - U_{max}) \cdot C_a$, where F is the perfusion rate (ml/min per g wet weight), U_{max} is the maximal fractional [^{14}C]methylaminoisobutyric acid uptake and C_a is the perfusate concentration of methylaminoisobutyric acid [2,3]. Pancreata were preperfused for 5 min with each designated concentration of methylaminoisobutyric acid to achieve equilibration of the extracellular space, and subsequently tracer amino acid uptake was measured. A single Michaelis-Menten kinetic analysis of α-methylaminoisobutyric acid influx indicated a $K_t = 1.7 \pm 0.2$ mM and $V_{max} = 0.49 \pm 0.03$ μmol/min per g (mean ± S.E., $n = 6$ pancreata). A low-affinity transport component was not detected in these experiments. Rat hepatocytes isolated from fed animals have a low affinity for aminoisobutyric acid ($K_t = 50–70$ mM), although a high-affinity transport component ($K_t = 0.6–0.8$ mM) completely inhibitable by methylaminoisobutyric acid emerges in the fasted state [14]. In hepatoma cells cultured in amino acid depleted medium the K_t for methylaminoisobutyric acid influx ranged between 0.1 and 0.2 mM [15], while K_t values between 0.2 and 0.3 mM have been reported in Ehrlich cells [16].

To determine whether entry of other amino acids was mediated via system A, experiments were undertaken to characterize the inhibitory effects of various amino acids. Influx of methylaminoisobutyric acid was measured at a constant perfusate concentration of 50 μM initially in the absence and then the presence of different inhibitor amino acids (10 mM, 20 mM or 40 mM). In four experiments we observed that during prolonged (60-min) perfusion with 50 μM methylaminoisobutyric acid influx decreased in a time-dependent manner with a maximum decrease of 23 ± 7% detected after 40-min perfusion. This may have been the consequence of trans-inhibition of extracellular amino acid uptake, a phenomenon well documented for system A [10,15]. Moreover, this hypothesis is supported by our observation that extracellular methylaminoisobutyric acid fails to trans-stimulate its own efflux from pancreata preloaded for 20 min with [^{14}C]methylaminoisobutyric acid, whereas many other amino acids are effective (Mann, G.E. and Norman, P.S.R., unpublished data). It seems unlikely that the viability of the pancreas was affected, since such a time-dependent decrease in influx was not observed in identical experiments with L-serine (see Fig. 1, Ref. 17).

Table I summarizes the inhibition of methylaminoisobutyric acid influx caused by substrates for different amino acid transport systems, and the inhibition data have been corrected for the time-dependent decrease in control influx. The

TABLE I

INHIBITION OF α-METHYLAMINOISOBUTYRIC ACID INFLUX BY DIFFERENT AMINO ACIDS

Influx of methylaminoisobutyric acid was measured at a constant perfusate concentration of 50 μM in the absence (J_c) and then the presence (J_i) of another unlabelled amino acid in the perfusate. Pancreata were preperfused for 5 min with a given solution before measuring the unidirectional methylaminoisobutyric acid influx. Following a control influx measurement, the inhibitory effect of four different amino acids was tested in random order in each pancreas. Methylaminoisobutyric acid (MeAIB) influx measurements were corrected for a time-dependent trans-inhibition of influx (see text) and the % inhibition in influx was calculated from % inhibition $= (1 - (J_i/J_c)) \times 100$. Inhibition values denote the mean ± S.E. of measurements in n perfused pancreata.

Amino acid	% Inhibition in MeAIB influx
10 mM methylaminoisobutyric acid	94 ± 0.7 (3)
40 mM DL-methylalanine *	96 ± 4 (3)
20 mM L-serine	91 ± 6 (3)
20 mM L-cysteine	89 ± 2 (3)
20 mM glycine	56 ± 2 (3)
20 mM L-phenylalanine	63 ± 8 (3)
20 mM L-glutamine	88 ± 5 (3)
20 mM L-lysine	42 ± 9 (3)
20 mM L-glutamate	20, 25 (2)

* Racemic mixture of D/L isomers.

small neutral amino acids L-serine and L-cysteine, as well as the *N*-methylated substrates DL-methylalanine and methylaminoisobutyric acid, significantly inhibited 50 μM methylaminoisobutyric acid influx. The smaller inhibition induced by glycine may reflect an alternative pathway previously described for glycine in pancreatic fragments [18,19]. The large neutral amino acids L-phenylalanine and L-glutamine were also effective inhibitors of influx, whereas the two charged substrates L-glutamate and L-lysine were less effective inhibitors. The wide tolerance of system A detected in the exocrine pancreatic epithelium is similar to that described in cultured hepatoma cells [20], which transport alanine, serine, cysteine, glutamine and phenylalanine by system A, and in Ehrlich cells [16] where the Na^+-dependent fraction of methionine transport seems to be mediated by system A and completely inhibits methylaminoisobutyric acid uptake. Further kinetic studies are necessary to determine whether the inhibitions observed in the pancreas are competitive. We previously reported that in the pancreas, as in Ehrlich cells [16] and hepatocytes [21], *N*-methylated analogues were ineffective inhibitors of L-phenylalanine [2] and L-glutamine [3] transport via the Na^+-independent large neutral system L. Moreover, in the present study methylaminoisobutyric acid (0.05–10 mM) had no effect on either the inward or outward fluxes of L-serine measured at 10 mM. In the exocrine pancreas L-serine is largely transported by a sodium-independent carrier [2] resembling system asc (preference for alanine, serine, cysteine) recently described in erythrocytes [22,23]. Although its transport would be expected to be relatively insensitive to inhibition by methylaminoisobutyric acid, L-serine was an effective inhibitor of methylaminoisobutyric acid influx (Table I). These findings suggest that in addition to transport via system asc a small fraction of serine influx may also be transported by the Na^+-dependent systems A (most amino acids but preference for small substrates with unbranched side chains) and ASC (preference for alanine, serine, cysteine).

The mechanisms by which Na^+ elevates organic substrate transport have been studied extensively since Crane's early report [24]. The driving force for accumulation of amino acids through system A appears to be dependent on the sodium electrochemical gradient [14,16,25,26], although in the case of glycine some studies have concluded that cellular ATP and not the Na^+ gradient drives amino acid accumulation [19,27]. In the absence of metabolic energy pancreatic membrane vesicles transport alanine against a concentration gradient at the expense of an inward directed Na^+ gradient [26]. Moreover, Na^+-amino acid cotransport has been shown to induce a marked depolarization of the basolateral membrane of the pancreatic acinar cell [28]. Since uptake of [^{14}C]methylaminoisobutyric acid was markedly inhibited in pancreata perfused with a Na^+-free solution [2], we have now attempted to investigate in more detail the effects of changing extracellular sodium concentration on methylaminoisobutyric influx measured at 50 μM and 100 μM. In these experiments isoosmolarity was maintained by replacing sodium chloride with choline chloride, and influx of methylaminoisobutyric acid was measured at different perfusate concentrations of sodium (26–156 mM). Arterial and venous Na^+ and K^+ concentrations were measured at regular intervals by flame photometry, however, we can only assume that the extracellular concentration of sodium approximated that of the arterial perfusate. Nevertheless, weighted Michaelis-Menten estimates revealed a sodium $K_t = 222 \pm 111$ mM ($n = 6$) and $K_t = 224 \pm 23$ mM ($n = 4$) at, respectively, 50 μM and 100 μM (Fig. 2A) methylaminoisobutyric acid concentrations. Equal confidence limits were obtained for linear regression analyses of double-reciprocal plots of 50 μM ($r = 0.81$) and 100 μM ($r = 0.61$) methylaminoisobutyric acid influx versus perfusate Na^+ (data not shown) and plots of influx against log perfusate Na^+ concentration (Fig. 2B). In most other isolated cell preparations the K_t of sodium for the carrier (Na^+ at half-maximal velocity) ranged between 10 and 60 mM [10,14, 20,21,25,26,29,30].

In rat liver membrane vesicles an increase in external alanine elevates the apparent K_t for sodium [31], and it is conceivable that in the pancreas raised methylaminoisobutyric acid concentrations may have altered the affinity of the carrier for sodium. When we attempted to determine the effects of sodium on methylaminoisobutyric acid influx at higher substrate con-

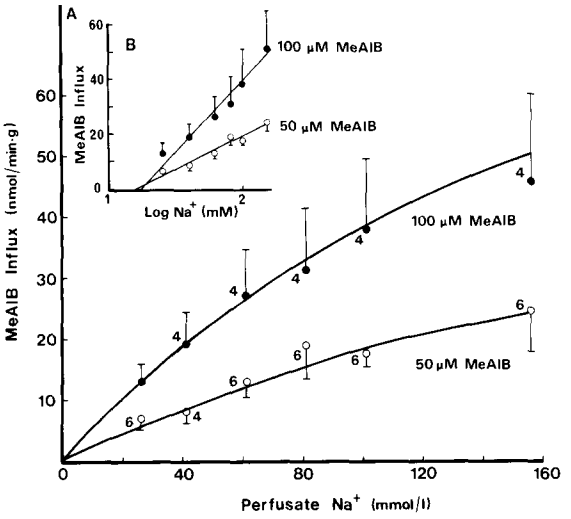

Fig. 2. Effects of lowering the extracellular sodium concentration upon the unidirectional influx of α-methylaminoisobutyric acid. (A) MeAIB influx (100 μM) was measured in isolated pancreata perfused in random order with different concentrations of sodium (26, 41, 61, 81, 101, 156 mM). NaCl was replaced with choline chloride. Influx was calculated as described in the text and is plotted against the perfusate sodium concentration. In these experiments perfusion pressure increased from 29±3 mmHg at 156 mM Na^+ to 36±5 mmHg at 26 mM Na^+. Michaelis-Menten and Lineweaver-Burk analyses of these data revealed K_t values for Na^+ in the region of 200 mM. (B) Linear relationship between MeAIB influx and the logarithmic perfusate sodium concentration. If the energy for MeAIB uptake were provided by the cation gradient one would expect transport to be proportional to the log Na^+ gradient and not the linear gradient [19]. As we did not measure the intracellular Na^+ concentration, we chose to plot MeAIB influx against the log extracellular Na^+ concentration. All values denote the mean ± S.E. of measurements in 4–6 perfused pancreata.

centration, kinetic analysis became difficult due to the low tracer amino acid uptake measured (18 ± 1% at 0.55 mM and 13 ± 1% at 2 mM). To our knowledge no other studies in perfused organs have attempted to characterize the kinetic relationship between extracellular sodium and amino acid influx. In the pancreatic epithelium methylaminoisobutyric acid influx via system A seems to be dependent on the extracellular sodium concentration (Fig. 2), and in preliminary experiments [17] influx was inhibited during perfusion with 1 mM ouabain but unaffected by 0.3 mM potassium. A linear relationship between 3-aminoendobicyclo(3,2,1)octane-3-carboxylic acid (BCO) transport and extracellular sodium has also been observed in mouse blastocytes and perhaps other undeveloped tissues [32] and for glycine transport in Ehrlich cells when the electrochemical sodium gradient was equilibrated by gramicidin treatment [27].

We have established that system A is fully expressed in the basolateral plasma membrane of the pancreatic epithelium, and that it behaves as a broad spectrum system tolerating a wide variety of other amino acids. It is highly dependent upon extracellular sodium, although the exact relationship between sodium, the amino acid analogue and carrier could not be fully identified in the present study. The existence of an insulo-acinar portal circulation in the pancreas [5] together with recent reports of adaptative regulation (enhanced transport activity) of system A in hepatocytes induced by amino acid starvation [14,15] suggest that the pancreas may be a useful model for further examining the endocrine and nutritional control of extracellular amino acid supply in secretory epithelia.

Financial support from Medical Research Council (U.K.) Project Grants G8223671SB and G8420750SB (G.E.M.) is gratefully acknowledged.

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