Biochimica et Biophysica Acta 858 (1986) 263–274
Elsevier

BBA 73104

Basolateral amino acid transport systems in the perfused exocrine pancreas: sodium-dependency and kinetic interactions between influx and efflux mechanisms

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(Received January 15th, 1986)

Key words: Amino acid transport; Na^+ dependence; Basolateral membrane; Kinetics; (Rat pancreas)

Basolateral amino acid transport systems have been characterized in the perfused exocrine pancreas using a high-resolution paired-tracer dilution technique. Significant epithelial uptakes were measured for L-alanine, L-serine, α-methylaminoisobutyric acid, glycine, methionine, leucine, phenylalanine, tyrosine and L-arginine, whereas L-tryptophan and L-aspartate had low uptakes. α-Methylaminoisobutyric acid transport was highly sodium dependent (81 ± 3%), while uptake of L-serine, L-leucine and L-phenylalanine was relatively insensitive to perfusion with a sodium-free solution. Cross-inhibition experiments of L-alanine and L-phenylalanine transport by twelve unlabelled amino acids indicated overlapping specificities. Unidirectional L-phenylalanine transport was saturable ($K_t = 16 \pm 1$ mM, $V_{max} = 12.3 \pm 0.4$ μmol/min per g), and weighted non-linear regression analysis indicated that influx was best described by a single Michaelis-Menten equation. The V_{max}/K_t ratio (0.75) for L-phenylalanine remainded unchanged in the presence of 10 mM L-serine. Although extremely difficult to fit, L-serine transport appeared to be mediated by two saturable carriers ($K_{t1} = 5.2$ mM, $V_{max1} = 7.56$ μmol/min per g; $K_{t2} = 32.8$ mM, $V_{max2} = 22.9$ μmol/min per g). In the presence of 10 mM L-phenylalanine the V_{max}/K_t ratio for the two L-serine carriers was reduced, respectively, by 79% and 50%. Efflux of transported L-[^{3}H]phenylalanine or L-[^{3}H]serine was accelerated by increasing perfusate concentrations of, respectively, L-phenylalanine and L-serine, and trans-stimulated by other amino acids. In the pancreas neutral amino acid transport appears to be mediated by Na^+-dependent Systems A and ASC, the classical Na^+-independent System L and another Na^+-independent System asc recently identified in erythrocytes. The interactions in amino acid influx and efflux may provide one of the mechanisms by which the supply of extracellular amino acids for pancreatic protein synthesis is regulated.

Introduction

Evidence from numerous studies in vitro indicates that the exocrine pancreas rapidly accumulates extracellular amino acids against a considerable concentration gradient [1–7]. The exocrine pancreas appears to metabolize amino acids in preference to D-glucose [5], and it is generally believed that the high transport rate for amino acids serves to provide the substrates for pancreatic protein synthesis [8–10]. Kinetic measurements obtained in mouse pancreatic fragments or slices incubated for 15–60 min suggested that neutral amino acid transport was mediated by

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several parallel carriers and that uptake was sensitive to extracellular sodium, ouabain and dinitrophenol [1–3]. Direct evidence for the existence of a sodium-coupled amino acid transport system was obtained in isolated pancreatic membrane vesicles [11] , and electrophysiological studies have shown that certain neutral amino acids including L-alanine depolarize the pancreatic acinar cell membrane by opening up conductance pathways primarily permeable to sodium [12,13]. As (Na^+-K^+)-activated ATPase has been localized in the basolateral plasma membranes of the pancreatic exocrine epithelium, it is conceivable that this enzyme may indirectly energize amino acid uptake by maintaining the Na^+-gradient [14]. Recent studies in the perfused exocrine pancreas have revealed that sodium-dependent neutral amino acid transport is stimulated by insulin and experimental diabetes [15].

The present experiments were undertaken to explore the specificity and concentration dependence of neutral amino acid influx and efflux at the basolateral membrane of the epithelium in the perfused rat exocrine pancreas. A preliminary account of part of this work has been communicated in abstract form [16].

Methods

Isolation and perfusion of the pancreas. Male Sprague-Dawley rats weighing 200–250 g were fasted for 24 h before being anaesthetized with intraperitoneal sodium pentobarbitone (60 mg/kg 'Sagatal'). Isolation of the pancreas entailed removing the stomach, spleen and intestine except for a small intestinal loop attached to the head of pancreas. As previously described [15,17], the pancreas was perfused in situ via the aorta (0.7–1 ml/min per g) with a Krebs-Henseleit bicarbonate solution containing 10 g/l bovine serum albumin (Cohn Fraction V, Sigma Chemical Co., U.K.). The pancreatic venous effluent either passed to waste or was collected sequentially from the cannulated portal vein.

The composition of the Krebs-Henseleit bicarbonate solution was (mM): NaCl, 118; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5; D-glucose, 5.5; and 1% bovine serum albumin. Perfusates were continually gassed at 38°C with 95% O_2 and 5% CO_2 to a pH between 7.3 and 7.4.

Amino acid uptake and efflux measurements. Amino acid transport at the basolateral plasma membrane of the pancreatic exocrine epithelium was studied using a rapid paired-tracer dilution technique [18]. Unidirectional tracer amino acid uptake was measured by directly comparing portal vein concentration profiles for a labelled amino acid and D-mannitol (extracellular tracer) following an intra-arterial injection (100 μl in 1–2 s) of perfusate containing both tracers. Usually thirty successive 60 μl venous samples were collected during 45–60 s. A final venous sample was accumulated for a further 4-min to assess tracer efflux from the pancreatic epithelium.

Tracer amino acid uptake was quantified in successive portal vein samples using the expression: uptake = 1–([^{3}H]amino acid/D-[^{14}C]mannitol). In the case of a ^{14}C-labelled amino acid D-[^{3}H]mannitol served as the extracellular reference tracer. Unidirectional amino acid influx was calculated from the equation: influx $= -F \cdot \ln(1 - U_{max}) \cdot C_a$, where F is the perfusion rate in ml/min per gram wet weight, U_{max} is the maximal fractional tracer amino acid uptake and C_a is the perfusate concentration of an unlabelled amino acid [15,17,18]. Efflux of a transported amino acid was estimated from: efflux $\% = (1 - (U_T/U_{max})) \times 100$, where the overall amino acid uptake (U_T) was calculated from the integrated tracer recoveries of the radioactive amino acid and D-mannitol starting from the time of maximal uptake and including the final 4-min accumulated venous sample (see Refs. 15, 17).

The cross-reactivity of different neutral amino acid transport systems was evaluated using two experimental designs: (i) In order to maximize amino acid uptake, transport was initially assessed during perfusion of the pancreas with an amino acid-free medium. Cross-inhibition tests of uptake were then delineated by adding twelve different unlabelled amino acids individually to the isotope injectates containing D-[^{14}C]mannitol and either L-[^{3}H]alanine or L-[^{3}H]phenylalanine. A 100 mM competitor amino acid concentration was used in the injectates, since dilution of the unlabelled amino acid should approximate the profile of the extracellular tracer D-mannitol (see Fig. 1 in Ref.

19) yielding a peak concentration of 4–5 mM at the exchange site. This protocol enabled us to effectively screen numerous interactions in each perfused pancreas during successive exposures to the tritiated amino acid and different unlabelled inhibitors. (ii) Kinetics of influx were studied under conditions in which pancreata were perfused with different concentrations of either L-phenylalanine (0.55–30 mM) or L-serine (0.05–50 mM). Preparations were equilibrated with each of 7 or 8 concentrations for 4 min before measuring unidirectional tracer amino acid uptake in the continued presence of substrate and each tracer injectate was made up to volume with the same solution perfusing the pancreas. Kinetic interactions between L-phenylalanine and L-serine transport were studied similarly by remeasuring influx kinetics for both amino acids in the presence of, respectively, 10 mM L-serine and 10 mM L-phenylalanine. The advantage of the latter experimental design is that concentration dependence of unidirectional influx and tracer efflux could be quantified simultaneously.

Radioactive molecules. The radioactive molecules L-[3-^{3}H]alanine (82.7 Ci/mmol), [^{14}C]methylaminoisobutyric acid (48.4 mCi/mmol), D-[1-^{3}H]mannitol (27 Ci/mmol) and D-[1-^{14}C]mannitol (45 mCi/mmol) were purchased from New England Nuclear Chemicals, Dreieich, F.R.G. L-[3-^{3}H]Serine (14 Ci/mmol), [2-^{3}H]glycine ((23 Ci/mmol), L-[*methyl*-^{3}H]methionine (85 Ci/mmol), L-[4,5-^{3}H]leucine (130 Ci/mmol), L-[4-^{3}H]phenylalanine (23.5 Ci/mmol), L-[3,5-^{3}H]tyrosine (52 Ci/mmol), L-[G-^{3}H]tryptophan (8.8 Ci/mmol), L-[5-^{3}H]arginine (8.5 Ci/mmol) and L-[2,3-^{3}H]aspartic acid (15 Ci/mmol) were obtained from Amersham International p.l.c., U.K.

Kinetic modelling analysis. All kinetic influx data obtained in the present experiments have been analyzed by weighted non-linear regression. Saturation, as well as, cross-inhibition kinetic data have been analyzed using single Michaelis-Menten, single Michaelis-Menten plus linear term and double Michaelis-Menten equations. Gardiner and Atkins [20] have reviewed details of these analyses, however, these authors stressed that "while parameters established by kinetic analysis are valid mathematical descriptions of the concentration-dependence of the uptake process, reliance must not be placed in them for interpretation of the actual mechanisms of transport".

Results

Unidirectional amino acid uptake and sodium-dependency

Basolateral amino acid transport sites in the exocrine epithelium of the perfused rat pancreas were characterized using a rapid paired-tracer dilution technique previously applied to the pancreas [15]. In order to maximize unidirectional amino acid uptake (relative to D-mannitol) measurements were initially made at tracer concentrations in preparations perfused with a sodium containing but amino acid-free (carrier-free) Krebs-Henseleit solution. The results in Fig. 1 show that uptake for tritiated L-phenylalanine, L-leucine and L-alanine was high during a single transit through the pancreas. A plateau maximal uptake was usually followed by efflux of the transported substrate from the epithelium into the circulation. The dotted lines in each panel of Fig. 1 illustrate the relationship between the maximal uptake (U_{max}) and the uptake determined in the final accumulated 4-min venous sample. The lower uptake in the 4-min venous sample indicates the presence of tracer efflux.

Table I summarizes the unidirectional uptakes measured for neutral, basic and acidic L-amino acids, as well as the model System A analogue α-methylaminoisobutyric acid. Epithelial uptake for the small neutral amino acids L-alanine and L-serine was approximately 60% whereas that for α-methylaminoisobutyric acid was 37%. The lower uptake measured for methylaminoisobutyric acid may reflect a decreased transport for this synthetic analogue and/or the low specific activity of the labelled substrate. High uptakes were also measured for L-methionine, the branched-chain amino acid L-leucine and the aromatic substrates L-phenylalanine and L-tyrosine. Uptakes for the protein-bound amino acid L-tryptophan, the cationic substrate L-arginine and the acidic substrate L-aspartate were considerably lower.

When sodium-dependency of neutral amino acid transport was examined, tracer amino acid uptake was initially measured during perfusion of pancreata with 143 mM sodium and then re-

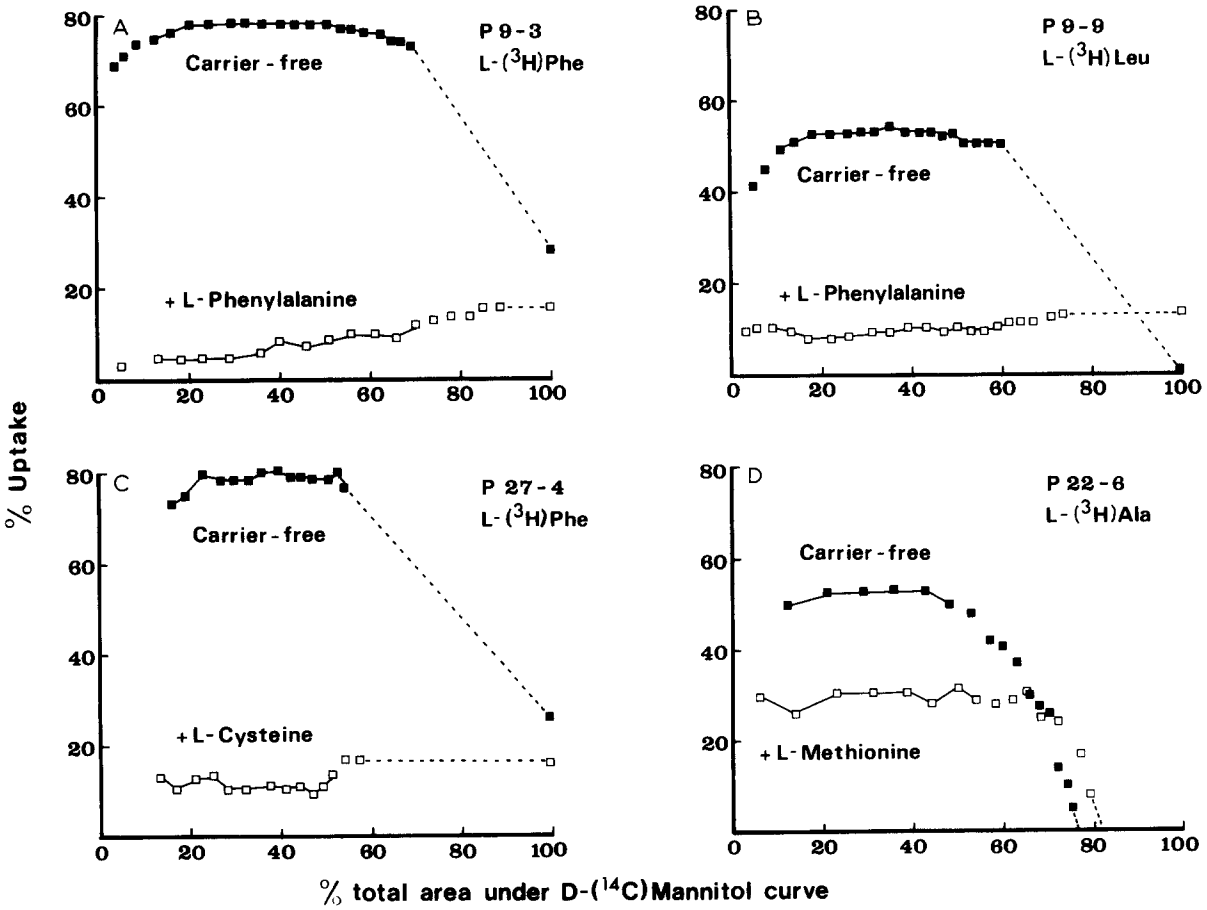

Fig. 1. Interactions between small and large neutral amino acid uptake in the exocrine pancreas. Tracer amino acid uptake was measured relative to D-[^{14}C]mannitol in pancreata perfused with an amino acid-free solution containing 5.5 mM D-glucose. The percentage uptake in successive venous samples has been weighed for the recovered reference tracer by plotting uptake values against the per cent accumulated area under the D-[^{14}C]mannitol dilution curve. Self- (graph A) and cross-inhibition (graphs B, C, D) tests of tracer amino acid uptake were performed by adding a specific unlabelled amino acid to an isotope injectate at a concentration of 100 mM. The unlabelled amino acid concentration was reduced to between 4 and 5 mM at the epithelial exchange site. Control patterns of uptake, indicated by the filled squares, were obtained in the same preparation as the inhibition tests. The dotted lines indicate the relationship between the maximal uptake measured during the single circulation through the pancreas (30 venous samples in 40–60 s) and the uptake measured in a final 4-min accumulated venous sample. Generally the high unidirectional uptake was followed by rapid tracer efflux from the epithelium.

measured 5-min after perfusing the same preparations with a sodium-free perfusate. Perfusion pressures remained constant throughout the total 10-min exposure to a sodium-free solution. As longer periods of perfusion with a Na^+-free solution led to marked increases in perfusion pressure, it seemed inappropriate to conduct detailed kinetic influx experiments in the absence of sodium. We were particularly interested in assessing the sodium-dependency of methylaminoisobutyric acid and L-serine uptake, since previous experiments in the pancreas had shown that transport of these substrates was insulin-sensitive [15]. The results in Table I reveal that uptake of methylaminoisobutyric acid was highly sodium-dependent, and moreover this transport inhibition was completely reversible upon reperfusion with a normal sodium containing perfusate. Surprisingly, uptake of L-serine was inhibited by only $18 \pm 3\%$. Transport of the larger neutral amino acids L-

TABLE I

UNIDIRECTIONAL AMINO ACID UPTAKE AT THE BASOLATERAL PLASMA MEMBRANE OF THE EXOCRINE PANCREATIC EPITHELIUM

Tracer amino acid uptake was measured during perfusion of pancreata with an amino acid-free Krebs-Henseleit bicarbonate solution. When the sodium-dependence of neutral amino acid uptake was determined, tracer uptake was initially measured during perfusion of pancreata with 143 mM Na^+ and then remeasured 5 min after switching to a perfusate in which NaCl and $NaHCO_3$ were replaced with buffered Trizma-HCl. The amino acid concentration in the different isotope injectates is tabulated. Values are mean ± S.E. (n = number of animals).

Amino acid	Injectate (μmol/l)	% Maximal uptake	% Sodium dependence [a]
L-[3-^{3}H]Alanine	0.4	59 ± 3 (12)	
L-[3-^{3}H]Serine	1.8	64 ± 2 (10)	18 ± 2 (5)
α-Methylaminoisobutyric acid	133	37 ± 2 (8)	81 ± 3 (3)
[2-^{3}H]Glycine	1.0	21 ± 0.3 (3)	
L-[*methyl*-^{3}H]Methionine	0.4	62 ± 7 (3)	
L-[4,5-^{3}H]Leucine	0.2	55 ± 3 (10)	11 ± 5 (3)
L-[4-^{3}H]Phenylalanine	1.0	71 ± 2 (13)	13 ± 6 (3)
L-[3,5-^{3}H]Tyrosine	0.7	57 ± 3 (3)	
L-[G-^{3}H]Tryptophan	3.8	11,12 (2)	
L-[5-^{3}H]Arginine	4.0	32 ± 2 (3)	
L-[2,3-^{3}H]Aspartic acid	2.0	11 ± 3 (3)	

[a] Sodium-dependent inhibition in uptake was reversible upon reperfusion with 143 mM Na^+.

leucine and L-phenylalanine was similarly sodium-independent. When we determined the sodium ion concentration in successive fractions of the pancreatic venous effluent, it was apparent that even 5-min after switching to a sodium-free perfusate the Na^+ concentration was still 7–11 mM. The washout profile for extracellular Na^+ was not altered significantly by longer pre-equilibration periods with a sodium-free solution.

Characterization of basolateral amino acid transport systems

Experiments were undertaken to determine whether uptake of neutral amino acids was mediated by one or more parallel transport systems. Similar to a previous study in the salivary epithelium [21], L-[^{3}H]alanine and L-[^{3}H]phenylalanine were selected as probe substrates for small and large neutral amino acid transport systems. Pancreata were perfused with a normal sodium but amino acid-free solution, and the inhibitory effect of numerous possible competitors was tested in each preparation by adding a different unlabelled amino acid (100 mM) to successive tracer injectates. Since the intracellular concentrations of L-alanine and L-phenylalanine in pancreata isolated from 24 h fasted rats are, respectively, 0.83 ± 0.06 mM and 0.14 ± 0.02 mM (Mann, G.E., Smith, S.A. and Emery, P.W., unpublished data), self-inhibition studies approximated zero-trans entry conditions. Moreover, the exocrine epithelium was only exposed to the tritiated test substrate and unlabelled inhibitor during the 45–60 s washout of the arterially injected 100 μl bolus. As shown in Fig. 1 L-phenylalanine caused a significant self-inhibition (graph A) and cross-inhibition of L-[^{3}H]leucine uptake (grap B). Further cross-inhibition tests revealed interactions between L-cysteine and L-phenylalanine (Fig. 1C) and L-methionine (a pancreatic imaging substrate) and L-alanine (Fig. 1D).

The inhibition of L-[^{3}H]alanine and L-[^{3}H]phenylalanine uptake by twelve different amino acids is summarized in Fig. 2. Transport of L-alanine was only sensitive to the L-enantiomer (50 ± 6%) and inhibited to a similar extent by L-cysteine (62 ± 8%). DL-Methylalanine, a substrate tolerated by System A, was tested at 200

mM but was a poor inhibitor of L-alanine uptake. The larger neutral amino acids L-methionine, L-leucine, L-phenylalanine and D-phenylalanine also caused a significant inhibition of L-[^{3}H]alanine and L-[^{3}H]phenylalanine transport. Unlike D-alanine, D-phenylalanine, L-cysteine and L-alanine were effective inhibitors of both L-alanine and L-phenylalanine uptake. L-Arginine and β-alanine had minimal effects on the transport of either test substrate. Glycine and hydroxy-L-proline had a negligible effect on L-phenylalanine uptake but inhibited L-alanine uptake by, respectively, 27 ± 7% and 20 ± 4%.

Two important findings were revealed in these injectate inhibition experiments; (i) an overlapping specificity in the uptake of small and large

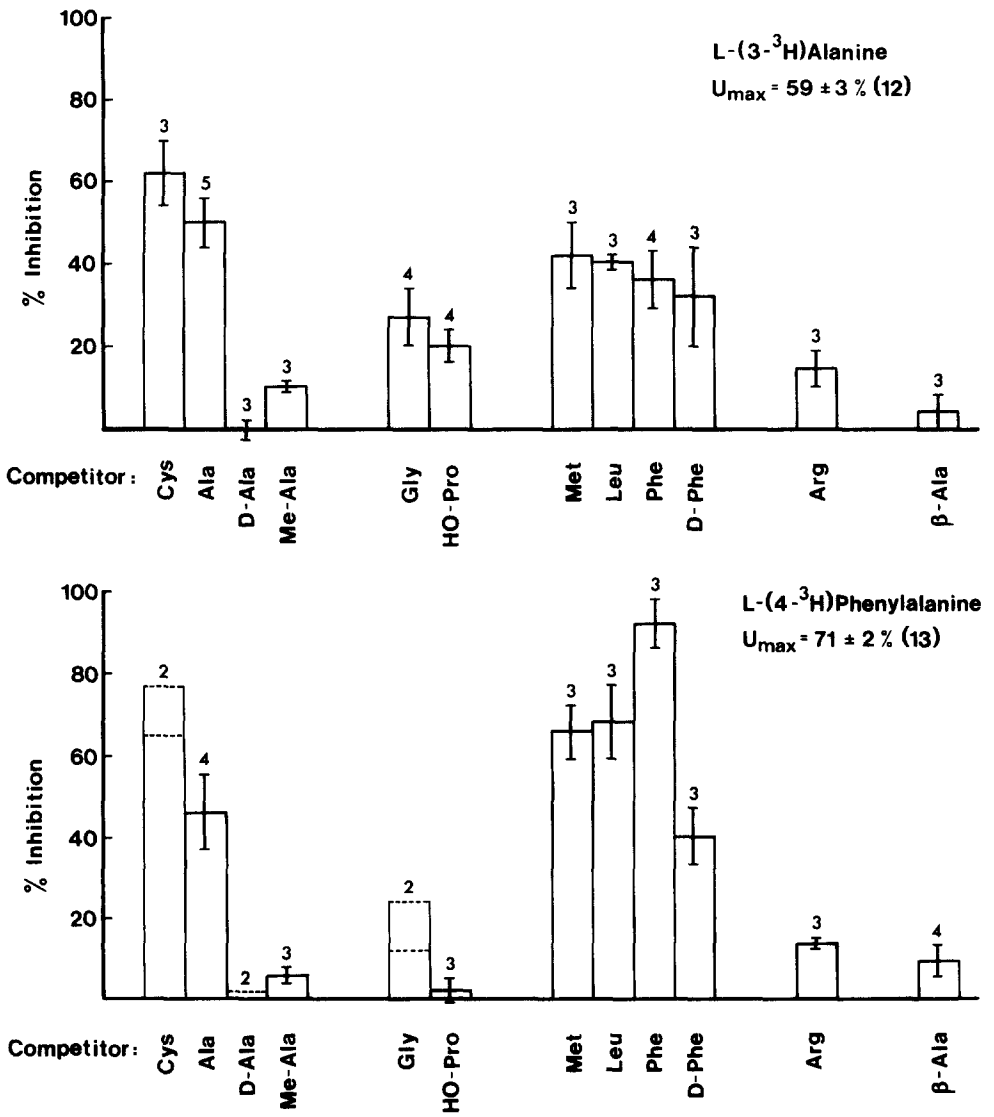

Fig. 2. Specificity of pancreatic neutral amino acid transport. Inhibition of L-[3-^{3}H]alanine and L-[4-^{3}H]phenylalanine uptake by 100 mM unlabelled L-amino acid competitors (unless specified) was tested as described in the legend to Fig. 2. The control unidirectional uptakes (U_{max}) measured for L-[^{3}H]alanine and L-[^{3}H]phenylalanine are specified in the right-hand corner of each panel. The percentage inhibition in U_{max} was calculated from U_{max} (control) − U_{max}(competitor)/U_{max}(control) × 100. Values are given as mean ± S.E., n = number of animals. Abbreviated amino acid nomenclature: Cys, cysteine; Ala, alanine; Me-Ala, DL-methylalanine; Gly, glycine; HO-Pro, hydroxypyroline; Met, methionine; Phe, phenylalanine; Arg, arginine; β-Ala, β-alanine.

neutral amino acids and (ii) a variable but general reduction in tracer efflux when transport of either L-[^{3}H]alanine or L-[^{3}H]phenylalanine was measured in the presence of an effective inhibitor (data not shown). The latter observation implies a cis-inhibition of tracer efflux by unlabelled small and large neutral substrates.

Kinetics and specificity of influx under 'steady-state' conditions

The design of the above experiments did not permit trans-stimulation of influx or efflux to be evaluted due to the brief exposure of the epithelium to test and competitor substrates. As uptake in vivo occurs from a relatively steady-state concentration of plasma amino acids, we decided to examine saturation and cross-inhibition kinetics of L-phenylalanine and L-serine influx in pancreata preperfused (in the presence of sodium) for 4 min with different concentrations of unlabelled substrate. In order to avoid problems associated with transamination of L-alanine, L-serine was selected as the small neutral test amino acid. L-Alanine and L-serine have the same maximal uptakes (Table I), appear to share the same transport site and moveover L-serine is stimulated by insulin and experimental diabetes [15].

As shown in the inset of Fig. 3, the maximal uptake for L-[^{3}H]phenylalanine was inhibited by increasing perfusate concentrations of unlabelled L-phenylalanine (0.55–30 mM). During continued perfusion with 5 mM L-phenylalanine tracer uptake was 42 ± 4% compared to 71 ± 2% measured from an amino acid-free solution (Table I). This 41% reduction in uptake is considerably less than the 92 ± 6% reduction determined in the injectate inhibition experiments (Fig. 2) where the exchange site concentration of L-phenylalanine approximated 4–5 mM. Unlike the injectate inhibition studies preperfusion with 5 mM L-phenylalanine may have induced trans-stimulation of L-[^{3}H]-phenylalanine uptake, a phenomenon characteristic of System L mediating the influx of large neutral amino acids. A single Michaelis-Menten analysis of phenylalanine transport revealed the lowest weighted standard deviation of residuals and indicated a $K_t = 16 \pm 1$ mM and $V_{max} = 12.3 \pm 0.4$ μmol/min per g (Figs. 3 and 4A, Table II).

In the presence of sodium L-serine transport was saturable at higher perfusate concentrations (0.05–50 mM), and kinetic modelling suggested entry via two parallel systems: $K_{t1} = 5.2$ mM, $V_{max1} = 7.56$ μmol/min per g and $K_{t2} = 32.8$ mM, $V_{max2} = 22.9$ μmol/min.g. An Eadie-Hofstee analysis of L-serine influx measured in the absence or presence of 10 mM L-phenylalanine in the perfusate indicate that L-serine transport was significantly inhibited by the aromatic analogue (Fig. 4B). As summarized in Table II, 10 mM L-phenylalanine lowered the V_{max}/K_t ratio for L-serine transport via these two systems by, respectively, 79% and 50%. When the kinetics of L-phenyl-

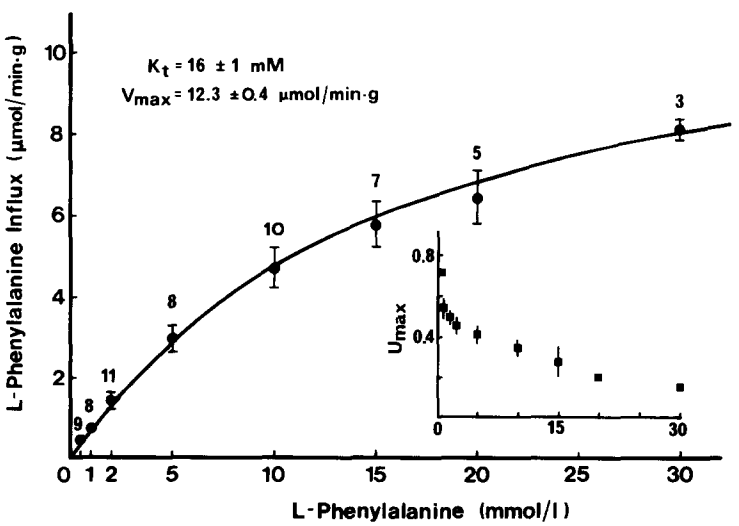

Fig. 3. Saturation kinetics for L-phenylalanine transport at the basolateral plasma membrane of the exocrine pancreatic epithelium. Unidirectional influx of L-phenylalanine was measured successively at six to eight different perfusate concentrations of unlabelled L-phenylalanine (0.55–30 mM). The solid curve represents a single rectangular hyperbola obtained by a direct fit to the mean influx values weighted for the reciprocal of their respective standard errors. The vertical lines denote the S.E. for at least three measurements from 3–11 different perfused pancreata. The inset illustrates that in these experiments L-[4-^{3}H]phenylalanine uptake decreased as the perfusate concentration of unlabelled L-phenylalanine was increased.

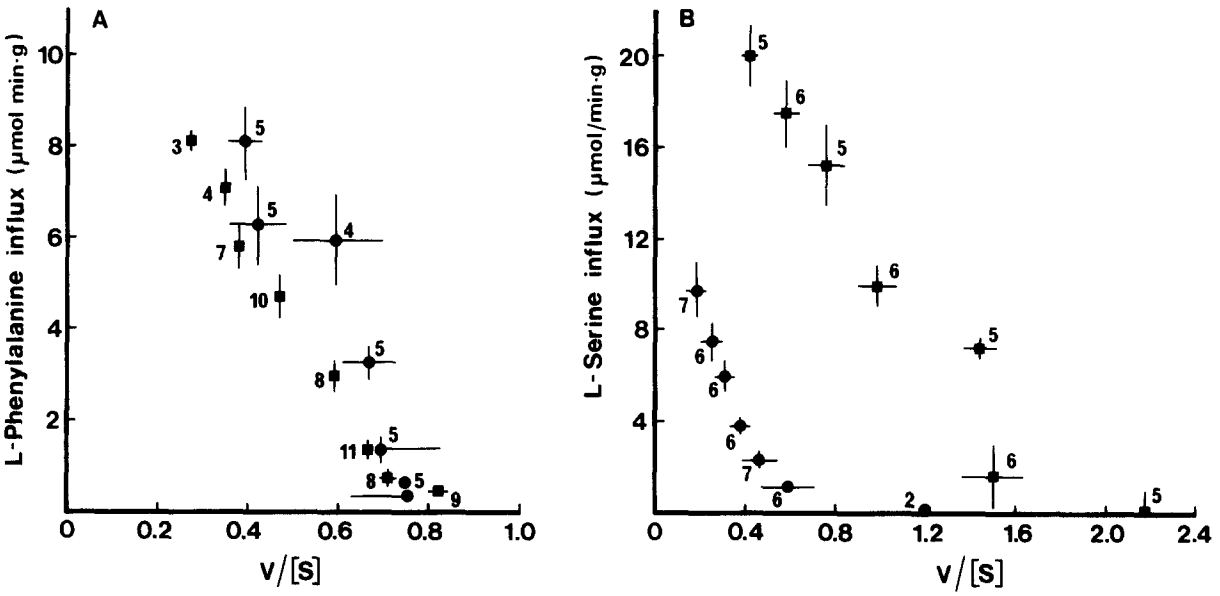

Fig. 4. Kinetic interactions in the transport of L-phenylalanine and L-serine. The kinetics of unidirectional L-phenylalanine (A) and L-serine (B) influx were measured over a wide range of perfusate concentrations. (A) L-phenylalanine transport (0.55–30 mM) was assessed in the absence (■———■, $n = 11$ pancreata) or presence of 10 mM L-serine (●———●, $n = 5$ pancreata). (B) L-serine transport (0.05–50 mM) was similarly measured in the absence (■———■, $n = 6$ pancreata) or presence of 10 mM L-phenylalanine (●———●, $n = 7$). The data are averages of influx determinations made in 5–11 perfused pancreata, and results are expressed as mean ± S.E. and have been plotted using an Eadie-Hofstee analysis.

TABLE II

KINETIC CONSTANTS FOR L-PHENYLALANINE AND L-SERINE TRANSPORT IN THE PERFUSED EXOCRINE PANCREAS

The procedures used to measure unidirectional influx kinetics for L-phenylalanine (0.55–30 mM) and L-serine (0.05–50 mM) are described in the legend to Figs. 3 and 4 and in the Methods. Kinetic cross-inhibition experiments were performed at fixed concentrations of 10 mM L-serine and 10 mM L-phenylalanine, respectively. A weighted non-linear regression analysis was used to model the kinetic data, and kinetic parameters were calculated from a minimum of 34 influx measurements obtained at seven perfusate concentrations. Values denote the mean ± S.E. of n animals in the case of a single Michaelis-Menten equation or the estimates obtained for a double Michaelis-Menten fit.

	n	K_t (mM)	V_{max} (μmol/min per g)	V_{max}/K_t
L-Phenylalanine				
Control	11	16.3 ± 1.1	12.3 ± 0.4	0.75
Control + 10 mM L-serine	5	18.6 ± 3.6	15.3 ± 1.8	0.82
L-Serine				
Control	6	14.4 ± 1.6	25.9 ± 1.4	1.80
Control + 10 mM L-phenylalanine	7	28.7 ± 2.7	15.0 ± 0.8	0.52

	n	K_{t1} (mM)	K_{t2} (mM)	V_{max1} (μmol/min per g)	V_{max2} (μmol/min per g)	V_{max1}/K_{t1}	V_{max2}/K_{t2}
L-Serine							
Control	6	5.2	32.8	7.56	22.9	1.45	0.70
Control + 10 mM L-phenylalanine	7	2.94	43.5	0.913	16.5	0.31	0.35

alanine transport were examined in the presence of 10 mM L-serine, the overall V_{max}/K_t ratio of 0.75 for phenylalanine remained unchanged (Fig. 4A, Table II). The resolution of these kinetic experiments suggests that L-serine has a low affinity for a large neutral System L, although L-phenylalanine and L-serine may share a similar low capacity entry system insensitive to Na^+.

Interactions in L-phenylalanine and L-serine efflux

Tracer efflux measurements were obtained simultaneously in the kinetic influx experiments summarized in Figs. 3 and 4 and Table II. Efflux of transported L-[^{3}H]phenylalanine (Fig. 5A) or L-[^{3}H]serine (Fig. 5B) was accelerated by increasing concentrations of the same unlabelled amino acid in the perfusate. During perfusion of pancreata with 20 mM L-phenylalanine approximately 94 ± 4% of L-[^{3}H]phenylalanine taken up returned to the circulation (Fig. 5A). By comparison only 81 ± 6% of transported L-[^{3}H]serine was recovered during perfusion with 50 mM L-serine (Fig. 5B). When the concentration dependence of L-[^{3}H]-phenylalanine efflux was assessed in the presence of 10 mM L-serine (Fig. 5A), it was apparent that at low substrate concentration (0.55 mM) efflux was increased from 50 ± 5% to 85 ± 5% (P = 0.001). This latter finding is of particular interest insofar as influx of L-phenylalanine was unaffected by L-serine (Fig. 4A and Table II). In the converse experiment 10 mM L-phenylalanine increased the efflux of L-[^{3}H]serine at low substrate concentrations (Fig. 5B).

Discussion

Amino acid transport studies performed in vitro with pancreatic fragments or acini have failed to

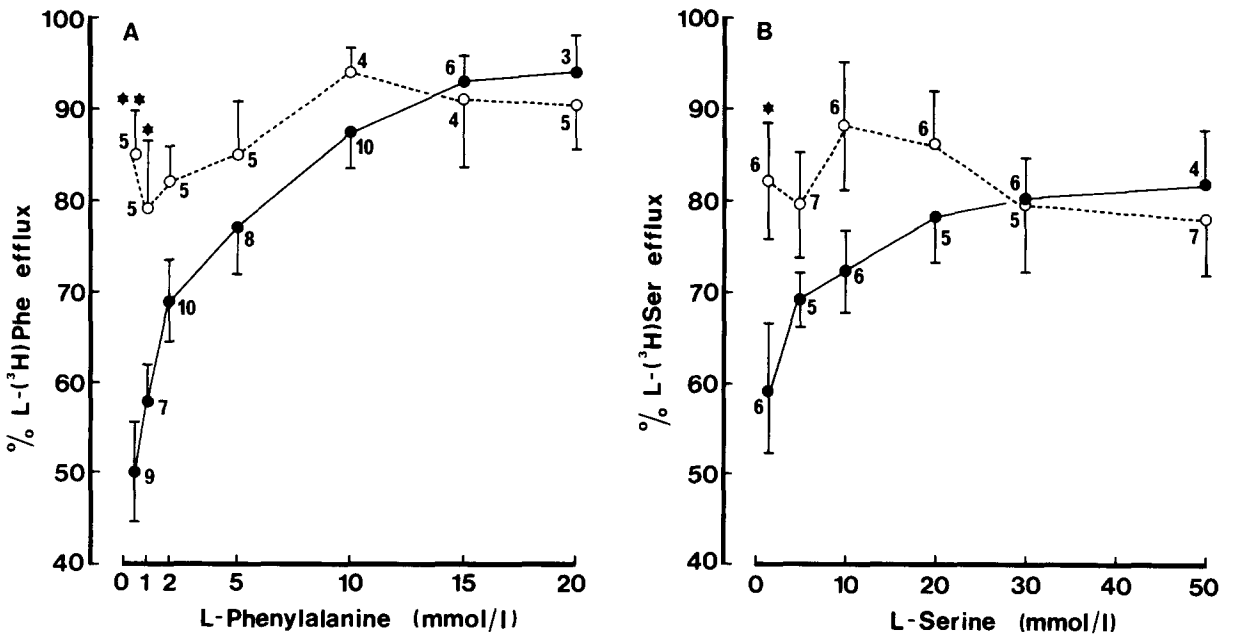

Fig. 5. Stimulatory effects of competitor amino acids on the concentration dependence of tracer amino efflux from the pancreatic epithelium. Kinetic influx and tracer efflux measurements were obtained simultaneously in the experiments summarized in Figs. 4 and 5 and Table II. Tracer amino acid efflux was estimated from $(1-U_T/U_{max})\times 100$, where the overall uptake of the tritiated amino acid (U_T) was calculated from the integrated tracer recoveries of D-[^{14}C]mannitol and the L-^{3}H-labelled amino acid starting from the time of maximal uptake (U_{max}) and including a final 4-min accumulated venous sample (see Ref. 15). (A) Concentration dependence of L-[4-^{3}H]phenylalanine efflux was determined in the absence (●———●, n = 11 pancreata) or presence (○———○), n = 5) of 10 mM L-serine in the perfusate. (B) Efflux of L-[3-^{3}H]serine was determined similarly in the absence (●———●, n = 6) or presence (○———○, n = 7) of 10 mM L-phenylalanine. Thin-layer chromatographic analyses [17] indicated that 5-min after the intraarterial injection of either L-[^{3}H]phenylalanine or L-[^{3}H]serine the ^{3}H activity was still associated with the native amino acid. ** P = 0.001, * P = 0.02 and 0.04.

demonstrate transport regulation by insulin or pancreatic secretagogues [5,22–24]. Our laboratory has recently demonstrated that in the perfused pancreas, as in many other tissues [25,26], neutral amino acid transport activity is stimulated by insulin [15]. The presenct study provides a detailed description of basolateral amino acid transport systems in the perfused rat exocrine pancreas in which the polarity of the basolateral and luminal poles of the epithelium was maintained. The time resolution of our experiments enabled us to identify interactions in the carrier-mediated influx and efflux of small and large neutral amino acids.

Specificity of basolateral amino acid influx mechanisms

The characteristics of different amino acid transport systems and the strategy for discriminating their transport kinetics have recently been reviewed [27]. In the pancreas significant uptakes were measured for the L-alanine, L-serine and the System A specific analogue α-methylaminoisobutyric acid in the presence of Na^+(Table I), however, methylalanine (another System A substrate) was a relatively poor inhibitor or L-alanine transport (Fig. 2). Moreover, in preliminary kinetic experiments α-methylaminoisobutyric acid at concentrations up to 10 mM caused no significant inhibition in the influx of 10 mM L-serine (Mann, G.E. and Norman, P.S.R., unpublished data). Further resolution of the entry mechanisms for these substrates was obtained when their sodium dependence was investigated. Uptake of α-methylaminoisobutyric acid was highly Na^+-dependent, whereas 82% of the total L-serine uptake was Na^+-independent (Table I). This residual Na^+-dependent fraction of L-serine uptake is similar to the fraction (0.10 ± 0.04) or L-alanine uptake inhibited by methylalanine (Fig. 2). In rat hepatocytes L-cysteine has been described as a model substrate for characterizing the Na^+-dependent System ASC [28,29], and neutral competitors including L-phenylalanine, proline and hydroxyproline have proved to be effective inhibitors of cysteine transport via System ASC [29]. The limited viability of the pancreas in sodium-free solutions prevented us from evaluating different competitors in the absence of sodium, a condition in which the activities of Systems A and ASC would be diminished. Based on the similar wide scope of interactions detected in inhibition studies of L-[^{3}H]alanine uptake in the pancreas (Fig. 2), it is conceivable that System ASC may also mediate a fraction of the small Na^+-dependent entry of L-serine.

Although transport of glycine has been evaluated extensively in the mouse pancreas in vitro [1–3], glycine uptake in the rat pancreas (Table I) was low ($21 \pm 0.3\%$) compared to that measured for L-alanine ($59 \pm 3\%$) and L-serine ($64 \pm 2\%$). It is of interest to recall that incubation of mouse pancreatic fragments in a sodium-free medium reduced indiscriminately the 60-min uptake (2 mM substrate concentration) of glycine, proline and cycloleucine by approximately 55% (see Table I, Ref. 1). In this same study 40 μM ouabain inhibited glycine uptake by 74% but cycloleucine uptake by only 52% (see Table II, Ref. 1). Even though we did not test the Na^+-dependence of glycine uptake, it seems reasonable to postulate, based on data obtained in the mouse and rat pancreas, that Na^+-dependent amino acid transport is mediated by more than one system.

In the presence of sodium L-serine was a poor inhibitor of L-phenylalanine influx, whereas L-phenylalanine significantly lowered the V_{max}/K_t ratio for L-serine transport (see Fig. 4A and Table II). These kinetic results, the sodium-independence (Table I) and ouabain-insensitivity [30] of L-serine uptake and the mutual trans-stimulation of efflux (Fig. 5) resemble a Na^+-independent System asc recently identified in horse erythrocytes by Fincham, Mason and Young [31] and pigeon erythrocytes by Vadgama and Christensen [32]. Its Na^+-independency and distinction from the Na^+-dependent System ASC is specified by the use of lower-case letters, a designation recently agreed upon by investigators involved in systems discrimination [33]. This novel high-affinity, stereoselective carrier also appears to be extremely sensitive to inhibition by *N*-ethylmaleimide [32]. However, a previous report from our laboratory demonstrated that L-serine influx at 10 mM was unaffected during perfusion of pancreata with 1 mM *N*-ethylmaleimide [15]. If we assume that in the pancreas L-serine and other neutral amino acids of intermediate size are largely transported

by an 'asc type' carrier, it would appear that this system may be modulated by exogenous insulin and insulin-sensitive diabetes mellitus (see Ref. 15).

The marked inhibition of L-phenylalanine uptake by L-methionine, L-leucine and L-phenylalanine (Fig. 2) and its lack of sensitivity to sodium (Table I) suggests the presence of another Na^+-independent carrier resembling the classical large neutral System L [34]. The cross-inhibitory effects observed in the uptake of small and large neutral amino acids (Fig. 2) and the reduction in L-serine influx induced by L-phenylalalanine (Fig. 4B) suggest a minor uptake of System L substrates by the 'asc type' system. The differential pH sensitivity of the Na^+-independent Systems L and asc detected in pigeon erythrocytes enabled Vadgama and Christensen [32] to resolve the scope of System asc, although they did not exclude minimal uptake of System L substrates by the pH-sensitive System asc. Perfusion of the intact pancreas at low pH for prolonged periods would lead to increases in vascular perfusion pressure, and hence it would be difficult to repeat the above experiments. Previous in vitro studies with rat pancreatic lobules used aminoisobutyric acid and L-leucine as model substrates for Systems A and L, and revealed that the calcium antagonist tetracaine selectively inhibited Na^+-dependent aminoisobutyric acid uptake [35]. Caution should be exercised in using aminoisobutyric acid as a model System A analogue since it is well known to interact with other neutral carriers.

Interactions in amino efflux

During perfusion of pancreata with an amino acid-free solution tracer efflux of L-phenylalanine or L-alanine was generally reduced in the presence of an injectate competitor. As discussed earlier, this may reflect cis-inhibition of efflux due to the absence of the native or competitor amino acid in the extracellular space following washout of the bolus injection. Tracer efflux was always accelerated in a concentration dependent manner by unlabelled substrate in the perfusate (Fig. 5). The mutual trans-stimulation of tracer L-phenylalanine and L-serine efflux by 10 mM L-serine and 10 mM L-phenylalanine together with the one-sided inhibitory effect of L-phenylalanine on L-serine influx provide further evidence for the activity of System asc [31,32]. In the mouse pancreas large neutral amino acids have been reported to undergo exchange diffusion, and the process appears to be independent of the presence of cations [36].

The importance of simultaneously assessing amino acid influx and efflux in our perfused pancreatic preparation is borne out by the observation that 72 h starvation modifies both the entry and exit mechanisms for L-phenylalanine [37]. In these 72 h fasted rats intracellular concentrations of the branched-chain and aromatic amino acids were significantly elevated [38] suggesting that the stimulation of influx may have been due in part to exchange diffusion. The present study provides a basis for evaluating the mechanisms underlying the endocrine regulation of extracellular amino acid supply in the normal and diabetic pancreas.

Acknowledgements

This work was financially supported by a Medical Research Council (U.K.) Project Grant G8223671SB to DR. G.E. Mann. A British Council/Spanish Government Academic Link (1981-1984) and Acciones Integradas Grant (1984-1985) provided travel and subsistence funds for exchange research visits between London and Malaga. We thank Dr. M. Munoz, Ms. Maria-Angeles Pastor and Mr. P.S.R. Norman for their assistance with some of these experiments. We are particularly grateful to Dr. M.L.G. Gardiner (School of Biological Sciences, University of Bradford), Dr. G.L. Atkins (Department of Biochemistry, University of Edinburg) and Dr. F.V. Sepulveda (AFRC Institute of Animal Physiology, Babraham) for their assistance in the kinetic modelling of our influx data.

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