Biochimica et Biophysica Acta, 1070 (1991) 135–142

BBAMEM 75456

Influx and incorporation into protein of L-phenylalanine in the perfused rat pancreas: effects of amino acid deprivation and carbachol

J.H. Sweiry [1], P.W. Emery [2], M. Muñoz [1]*, K. Doolabh [1] and G.E. Mann [1]

[1] *Biomedical Sciences Division, King's College London, London (U.K.) and*
[2] *Department of Nutrition, King's College London, London, (U.K.)*

(Received 8 April 1991)

Key words: Exocrine pancreas; L-Phenylalaline; Amino acid transport; Protein synthesis; Carbachol; (Rat)

The rate of protein synthesis in the isolated perfused rat pancreas was measured from the rate of incorporation of L-[^{3}H]phenylalanine into total protein, and was compared with the transport of this amino acid into the epithelium. Unidirectional (15 s) and net (15–30 min) uptake of L-[^{3}H]phenylalanine was measured relative to D-[^{14}C]mannitol (extracellular marker) using a cell loading technique. The fractional rate of protein synthesis in the pancreas was also measured *in vivo* using a flooding dose technique and found to be 118 ± 10% day^{-1} (corresponding to an absolute rate of incorporation of L-Phe into protein of 36.1 ± 3 nmol min^{-1} g^{-1}) in overnight fasted rats. Compared with the *in vivo* rate, the perfused pancreas exhibited a markedly lower rate of protein synthesis which increased significantly when amino acids were added to the perfusate (15.6 ± 1.9 vs. 22.5 ± 0.9% day^{-1} or 4.7 ± 0.6 vs. 6.9 ± 0.3 nmol L-Phe min^{-1} g^{-1}). Carbachol ($3 \cdot 10^{-7}$ M) stimulated protein synthesis provided amino acids were also supplied in the perfusate. Protein synthesis rates measured under all conditions *in vivo* and *in vitro* were at least an order of magnitude lower than the unidirectional influx (121 ± 14 nmol min^{-1} g^{-1}) of L-phenylalanine into the pancreatic epithelium. These results demonstrate that amino acid transport across the basolateral membrane of the epithelium is not rate-limiting for pancreatic protein synthesis.

Introduction

Synthesis and secretion of pancreatic enzymes appears to be under dietary [1–5] and hormonal control [6–10]. Although both dietary manipulation and treatment with secretagogues cause parallel and nonparallel changes in the rates of synthesis of different pancreatic enzymes both *in vivo* and *in vitro*, there is little information on the absolute rates of pancreatic protein synthesis. Fractional synthesis rates of up to 290% day^{-1} were reported in overnight fasted rats [11] and 185% day^{-1} in fed 4-week-old rats [12], while in the developing ovine pancreas synthesis rates ranged between 144–244% day^{-1} depending on age [13]. These findings indicate that the fractional rate of protein synthesis in the pancreas is amongst the highest in any mammalian tissue. Moreover, many studies have shown that under appropriate conditions cholecystokinin (CCK), or its analogue caerulein, induces a marked increase in the rate of pancreatic protein synthesis [6,7,9,14]. It is thus theoretically possible that substrate supply could become rate-limiting for protein synthesis, and that the influx of amino acids into the pancreatic epithelial cell could modulate the rate of protein synthesis.

We have previously shown that undirectional transport of amino acids, including L-phenylalanine, into the rat exocrine pancreatic epithelium is carrier-mediated and sensitive to dietary manipulations [15,16]. This study examines whether influx of L-phenylalanine into the exocrine pancreas is rate-limiting for total protein synthesis by measuring both processes simultaneously

* Present address: Departamento de Bioquimica y Biologia Molecular, Facultad de Medicina Universidad de Malaga, Malaga, Spain.

Correspondence: G.E. Mann, Physiology, Biomedical Sciences Division, King's College London, Campden Hill Road, London W8 7AH, U.K.

in the isolated perfused rat pancreas. For comparative purposes we also measured the rate of pancreatic protein synthesis *in vivo* in a matched group of rats. Preliminary findings of part of this work have been published in abstract form [17].

Methods

Isolation and perfusion of the pancreas. Male Sprague-Dawley rats weighing 210–260 g were allowed free access to water and a standard laboratory diet (No. 491, Grain Harvesters Ltd., Kent, U.K.) Animals were fasted overnight and anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg Sagatal). Intact pancreata, including a small section of the duodenum (previous experiments have shown that amino acid transport rates are not significantly different in pancreatic preparations with or without the small duodenal section (Norman, P.S.R. and Mann, G.E., unpublished observations)), were perfused at constant flow (about 1.8 ml/min) via the superior mesenteric and coeliac arteries with the portal vein as outlet [15,16,18]. The hepatic and common bile duct were ligated, and pancreatic juice was collected from the duodenal end of the cannulated main duct [18]. Isolated pancreata were maintained in a temperature-controlled tissue bath and perfused with a heated (37 °C), Krebs-Henseleit bicarbonate solution (gassed with 95% $)_2$/5% CO_2 to a pH of 7.35–7.45) of the following composition (mM): NaCl, 131; KCl, 5.6; $CaCl_2$, 2.5; $MgCl_2$, 1.0; NaH_2PO_4, 1.0; $NaHCO_3$, 25; D-glucose, 2.5; L-phenylalanine, 0.1; Dextran T70 (5% w/v, Meito Sangyo Co., Japan). In some experiments an amino acid mixture (5.16 mM) prepared in our laboratory was added to the perfusate to reproduce the plasma composition of individual amino acids as measured previously in fed animals [19]. The composition of this amino acid mixture was (mM): Arg, 0.223; His, 0.105; Ile, 0.107; Leu, 0.163; Lys. 0.347; Met, 0.067; Phe, 0.102; Thr, 0.158; Val, 0.238; Ala, 0.777, Cys, 0.036; Glu, 0.176; Asp, 0.052; Gln, 0.863; Gly, 0.473; Ser, 0.273; Tyr, 0.076, Tau, 0.354; Cit, 0.139; Asn, 0.083; Trp, 0.047; Pro, 0.3. In other experiments pancreata were perfused with carbachol ($3 \cdot 10^{-7}$ M), an analogue of the pancreatic secretagogue acetylcholine, to induce pancreatic secretion.

Unidirectional and steady-state uptake of amino acids. Unidirectional (15 s) and steady-state L-phenylalanine uptake was measured at a substrate concentration of 0.1 mM using a dual tracer loading technique, which has previously been applied to the perfused rat pancreas [20]. After 20 min pre-equilibration, pancreata were perfused from a reservoir to which D-[^{14}C]mannitol (0.05–0.09 μCi/ml) and L-[^{3}H]phenylalanine (0.5–0.9 μCi/ml giving a final specific activity of 5–9 μCi/μmol) were added, and the portal vein effluent was immediately sampled (100 μl) at rapid intervals (25 samples in < 1 min) followed by 1 min intervals for 5, 15 or 30 min. Tracer concentrations in the venous samples were expressed as a percentage of the activity (cpm/ml) in the arterial reservoir and L-[^{3}H]phenylalanine uptake (U) was measured in successive samples from: $U = 1 - (\%\ [^3H])/(\%\ [^{14}C])$. During the loading period L-[^{3}H]phenylalanine uptake decreased steadily, due to progressive tracer efflux tracer from the epithelium, and approached a steady-state in which there was a small net uptake (see Fig. 1). Unidirectional fractional amino acid uptake (U_{max}, see Fig. 1, inset), measured over the first 15–30 s was used to calculate influx (ν) from

$$\nu = [-F \cdot \ln(1 - U_{max}) \cdot C_a]$$

where C_a is the perfusate phenylalanine concentration (0.1 mM) and F the perfusion rate in ml/min per g [21]. Steady-state L-phenylalanine transport was measured similarly from the averaged uptake value after 15 min of cell loading (Fig. 1).

Estimation of pancreatic juice outflow and amylase activity. Basal and carbachol-stimulated pancreatic secretion was collected using a calibrated silicon tube (0.5 mm o.d.) attached to the free end of a cannula inserted into the main exocrine duct [18]. The tubing was replaced at 5-min intervals and the pancratic juice content in the tube was measured. The α-amylase content was assayed by using a standard kit from Pharmacia Diagnostics (Sweden). Under basal conditions there was a small but consistent pancreatic juice flow with little amylase activity. Upon continuous perfusion with $3 \cdot 10^{-7}$ M carbachol there was a marked stimulation of pancreatic juice flow and amylase output (data not shown).

Protein synthesis in perfused pancreas. Perfusion with L-[^{3}H]phenylalanine was maintained for 5, 15 or 30 min and the pancreas was then rapidly separated from the remaining small section of duodenum and immediately frozen in liquid nitrogen and stored at −20 °C for subsequent analysis. Pancreatic tissue was homogenised in 2% perchloric acid at 4 °C and free amino acids were separated as the supernatant after centrifugation. Precipitated proteins were washed three times with 2% perchloric acid and hydrolysed in 6 M hydrochloride acid at 105 °C for 18 h. The specific radioactivity of phenylalanine in hydrolysates, free amino acids and arterial perfusate samples was measured by the method of Garlick et al. [22]. Briefly this consists of enzymatic conversion of phenylalanine to β-phenylethylamine, extraction of β-phenylethylamine into heptane/chloroform (3:1) at alkaline pH followed by back extraction into 0.01 M sulphuric acid.

Radioactivity was measured in one aliquot by scintillation counting and β-phenylethylamine content was measured in another aliquot by the fluorometric assay of Suzuki and Yagi [23].

The fractional rate of protein synthesis (K_s) was calculated from:

$$K_s = (SA_{protein} / \overline{SA}_{free}) \cdot t \quad (1)$$

where $SA_{protein}$ is the final specific radioactivity of L-phenylalanine in pancreatic protein, $\overline{SA}_{free}$ is the average specific radioactivity of free L-phenylalanine in the precursor pool, and t is the time of incorporation. In these experiments $\overline{SA}_{free}$ was taken as equal to the final specific radioactivity of L-phenylalanine in the acid soluble fraction of the tissue, since we found rapid equilibration between the intracellular pool and the perfusate (see Fig. 3).

Protein synthesis in vivo. Rats were fasted overnight and anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg Sagatal). L-[^{3}H]phenylalanine (150 μmol and 50 μCi per ml per 100 g) was then injected via a lateral tail vein, and after 5 or 15 min the peritoneum was opened, a sample of blood was taken from the abdominal aorta and the pancreas was removed, rapidly cleared of the surrounding fat and frozen in liquid nitrogen. After storage at −20 °C the plasma and pancreas samples were analyzed for specific radioactivity of free and protein-bound L-phenylalanine as described for the perfusion experiments.

The specific radioactivity of intracellular free phenylalanine was found to fall from a mean value of 678 ± 16 (n = 6) dpm/nmol at 5 min to 536 ± 12 (n = 6) dpm/nmol at 15 min. The rate of protein synthesis was therefore calculated for the rats killed at 15 min from Eqn. 1, taking SA_{free} (mean precursor specific activity over 15 min) as 1.20 times the final intracellular free phenylalanine specific activity.

Amino acid analysis. Perchloric acid soluble supernatants from the pancreas homogenates were neutralised with saturated tripotassium citrate and analyzed for amino acid composition by high performance liquid chromatography, using the method described previously [19].

Radioactive molecules and other materials. L-Phenyl[2,3-^{3}H]alanine (45 Ci/mmol) and D-[1-^{14}C]mannitol (53.4 mCi/mmol) were obtained from NEN, Dreieich, F.R.G. All amino acids and carbachol were of the highest grade and were purchased from Sigma Chemical Co., U.K.

Statistics. All values are expressed as mean ± S.E. where n indicates the number of animals. Statistical analyses were performed using analysis of variance and student's unpaired t-test; values of $P < 0.05$ were considered significant.

Results

Undirectional and net uptake of L-phenylalanine

Fig. 1 illustrates a typical dual-isotope experiment in which both unidirectional and steady-state L-phenylalanine uptake by the pancreatic epithelium were measured. During 30 min perfusion with D-[^{14}C]mannitol and L-[^{3}H]phenylalanine, the radioactivity of D-[^{14}C]mannitol (extracellular marker) in the venous perfusate rapidly approached that in the arterial perfusate, whereas the recovery of L-[^{3}H]phenylalanine in venous samples was relatively much lower than that of the extracellular marker. This difference in the venous concentrations-time profiles represents L-[^{3}H]phenylalanine uptake by the exocrine pancreas. The maximal uptake (U_{max}, about 70%) occurred within seconds (Fig. 1 inset) and was followed by rapid tracer efflux leading to steady-state uptake values of a few percent after 15–30 min.

Effects of amino acids and carbachol on L-phenylalanine transport

In preparations perfused in the absence of amino acids (except 0.1 mM L-phenylalanine) and carbachol the measured maximal uptake (71 ± 4%) corresponded to a unidirectional influx of 162 ± 6 nmol/min per g (n = 7). Net uptake (4 ± 1%) measured between 15 and 30 min corresponded to a net transport of 5.0 ± 1.4 nmol/min per g (n = 3). The addition of a mixture of amino acids (5.16 mM), equivalent to plasma concen-

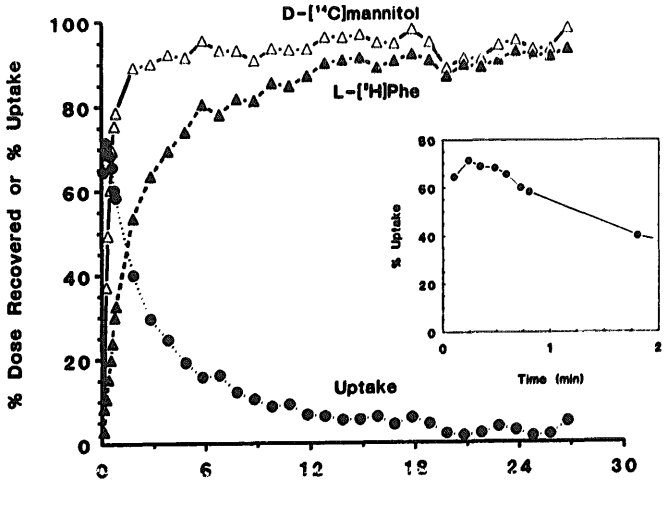

Fig. 1. Unidirectional and net uptake of L-[^{3}H]phenylalanine in the perfused rat pancreas. The ordinate represents the venous effluent recovery of radioactivity, expressed as a percentage of the respective arterial reservoir cpm/ml for L-[^{3}H]phenylalanine (▲) and D-[^{14}C]mannitol (△, extracellular marker). Uptake (U) of L-[^{3}H]phenylalanine (●) was calculated from $U = 1 - \{[^3H \text{ activity}]/[\text{D-}[^{14}C]\text{mannitol activity}]\}$ in successive venous samples. The inset shows % uptake measured during the first 2 min (expanded scale) of cell loading. In this experiment the perfusate was free of amino acids and carbachol.

TABLE I

Effects of carbachol ($3 \cdot 10^{-7}$ M) and extracellular amino acids (5.16 mM) on L-phenylalanine transport in the perfused rat pancreas

Unidirectional influx and net transport were measured over the first 15–30 s and over the steady-state period (20–30 min, see Fig. 2), respectively. Results are mean ± S.E. of n pancreata in each group. Significance of difference by unpaired t-tests: [a] $P < 0.03$ compared with amino-acid-free and carbachol(CCh)-free group; [b] $P < 0.01$ compared with amino-acid-free and CCh-free group.

	Amino-acid-free		+ Amino acids	
	CCh-free	+ CCh	CCh-free	+ CCh
Influx (nmol/min per g) (n)	162 ± 6 (7)	127 ± 6 [a] (3)	121 ± 14 [b] (6)	127 ± 16 (6)
Net transport (nmol/min per g) ($n = 3$)	5.0 ± 1.4	− 1 ± 0.8 [b]	5.4 ± 1.2	6.9 ± 1.5

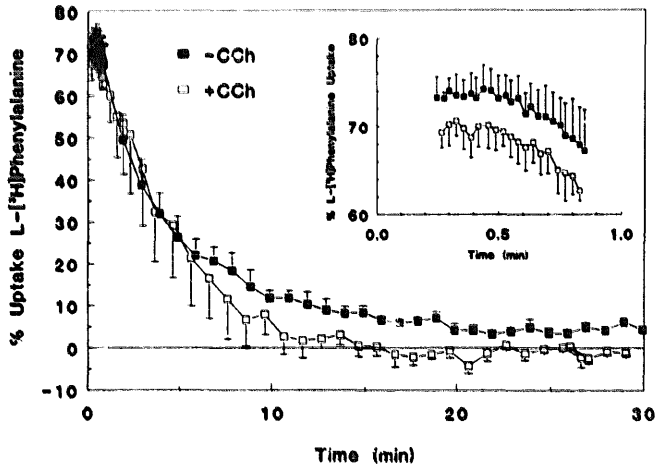

Fig. 2. Effect of carbachol on unidirectional and net uptake of L-phenylalanine. The inset illustrates % uptake measured during the first 1 min (expanded scale) of cell loading. In these experiments pancreata were perfused with an amino acid-free solution in the absence or presence of $3 \cdot 10^{-7}$ M carbachol which was perfused continuously starting 20 min prior to uptake measurements. Values are mean ± S.E. of three preparations in each group.

TABLE II

Time course of specific (SA) of L-phenylalanine pools and rates of protein synthesis (K_s) in the perfused pancreas

Results are mean ± S.E. of three perfused with an amino-acid-free and carbachol-free medium.

	SA_{free} (dpm/nmol)	$SA_{protein}$ (dpm/nmol)	K_s (% day^{-1})
5 min	19963 ± 1396	14.0 ± 2.9	19.9 ± 3.3
15 min	19343 ± 1894	34.2 ± 5.9	16.7 ± 1.4
30 min	20237 ± 323	65.7 ± 8.0	15.6 ± 1.9

trations, to the perfusate significantly reduced unidirectional influx by about 25%, but had no effect on net uptake (Table I). This presumably reflects competition by some of these amino acids for the L-phenylalanine carrier [24].

Fig. 2 illustrates that continuous perfusion with $3 \cdot 10^{-7}$ M carbachol (over 30 min), in the absence of amino acids, resulted in a reduction in the unidirectional and net uptake of L-[^{3}H]phenylalanine. In contrast carbachol had no effect on L-phenylalanine transport during perfusion with amino acids (Table I).

Pancreatic protein synthesis

The specific activities of free and protein-bound L-phenylalanine in pancreata perfused with amino acid-free medium are shown in Table II. Fractional rates of total protein synthesis calculated from these data remained unchanged ($P > 0.05$, one way analysis of variance) after 5, 15 or 30 min of perfusion of isolated pancreata. In subsequent experiments measurements were made at 5 and 30 min, but since the results were similar only the data obtained at 30 min will be discussed.

TABLE III

Specific activities (SA) of L-phenylalanine pools, rates of protein synthesis (K_s) and rates of L-phenylalanine incorporation into total protein in the isolated perfused pancreas and in vivo

Results are mean ± S.E., $n = 3$ pancreata in perfusion experiments and 6 pancreata *in vivo*. Carbachol (CCh) concentration was $3 \cdot 10^{-7}$ M. Significance of differences in K_s in 30 min perfusion experiments by two way analysis of variance: effect of amino acids, $P < 0.001$; effect of CCh, P n.s.; interaction, $P < 0.01$.

	SA_{free} (dpm/nmol)	$SA_{protein}$ (dpm/nmol)	K_s (% day^{-1})	Incorporation into protein (nmol L-Phe/min per g)
Perfused pancreas				
Amino-acid-free:				
CCh-free	20237 ± 323	65.7 ± 8.0	15.6 ± 1.9	4.7 ± 0.6
+ CCh	15335 ± 1078	35.9 ± 1.1	11.3 ± 0.7	3.5 ± 0.2
+ Amino acids:				
CCh-free	15345 ± 1679	72.6 ± 10.5	22.5 ± 0.9	6.9 ± 0.3
+ CCh	10917 ± 125	65.8 ± 2.14	28.9 ± 1.1	8.9 ± 0.3
Pancreas *in vivo*	536 ± 12	7.92 ± 0.66	118 ± 10	36.1 ± 3.0

Effect of amino acid supply and carbachol on pancreatic protein synthesis

Previously in our laboratory most transport studies have been performed in the absence of other amino acids in the perfusate in order to maximise tracer uptakes. Thus, pancreatic protein synthesis in this study was initially measured under these conditions. However, it was also important to examine the effects of added extracellular amino acids on protein synthesis. Table III shows that the presence of a mixture of amino acids almost doubled the rate of protein synthesis. Perfusion with carbachol ($3 \cdot 10^{-7}$ M) in the presence, but not in the absence, of amino acids further increased the rate of total protein synthesis by about 30%.

The maximum fractional rate of protein synthesis measured in the isolated perfused rat pancreas was $28.9 \pm 1.1\%$ day^{-1} (Table III). If we assume that the pancreas contains 15% protein, and that pancreatic protein contains 49.6 mg L-phenylalanine per g protein Emery, P.W., unpublished observations), this corresponds to incorporation of L-phenylalanine into protein at a rate of 8.9 ± 0.3 nmol/min per g ($n = 3$). This is at least an order of magnitude lower than the unidirectional influx (Table I).

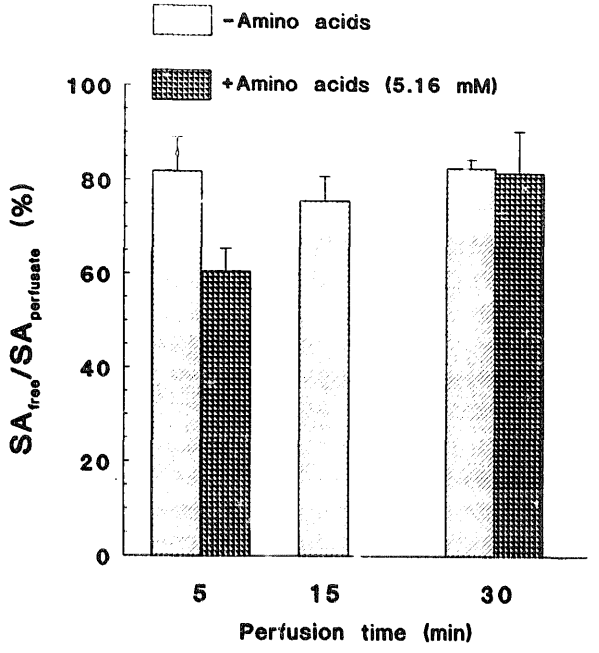

Fig. 3. Time course of equilibration of L-phenylalanine specific activity between the intracellular free amino acid pool and perfusate. The ratio of specific activities (SA) between the pools is shown for two of the conditions studied in perfusion experiments *in vitro*. Unlabelled L-phenylalanine was always present at 0.1 mM and carbachol was absent. Data are mean ± S.E. of three experiments in each group.

In vivo protein synthesis

For comparative purposes we also measured the rate of protein synthesis *in vivo*, and Table III shows that this was about 5-fold greater than that measured *in vitro* in the perfused pancreas, in the presence of amino acids. The fractional rate of $118 \pm 10\%$ day^{-1} corresponds to L-phenylalanine incorporation at a rate of 36.1 ± 3.0 nmol/min per g. This is still considerably lower than the unidirectional influx.

Precursor pool for protein synthesis

Fig. 3 shows that the specific activity of intracellular free L-phenylalanine in the perfused pancreas, expressed as a proportion of the arterial perfusate specific activity, rose rapidly, reaching a value at 5 min which did not change significantly after 15 or 30 min during perfusion with an amino acid-free medium. The presence of a mixture of other amino acids in the perfusion medium slowed the attainment of isotopic equilibrium in the first 5 min, again presumably because of competition for carrier sites. The addition of carbachol did not significantly affect the isotopic equilibrium between the intracellular free amino acid pool at any of the time points measured (data not shown). The intracellular free L-phenylalanine specific activity at 30 min was 82–88% of the corresponding arterial perfusate value for all conditions tested. Thus, if the true precursor pool for protein synthesis is actually extracellular our calculations based on the intracellular specific activity would overestimate the true rate of protein synthesis by about 12–18%.

The attainment of isotopic equilibrium between the pancreas and the plasma *in vivo* was also rapid, and did not change significantly between 5 ($84.7 \pm 1.3\%$) and 15 ($79.8 \pm 0.4\%$, $n = 6$) min.

Concentrations of free amino acids

In an attempt to correlate the protein synthetic rates measured *in vitro* with the amino acid content of pancreatic tissue we measured the concentration of a range of amino acids in the perfused pancreata and *in vivo*. Table IV shows the concentrations of free amino acids in pancreata at the end of 30 min perfusion with labelled L-phenylalanine, and *in vivo* at the time (15 min following the injection of L-[^{3}H]phenylalanine) of measuring the rate of protein synthesis. Perfusion with amino acid-free medium caused a 60% reduction in total amino acid concentration in comparison with pancreata perfused with plasma concentrations of amino acids and this may account for the reduced rate of protein synthesis rates. Pancreatic concentrations of phenylalanine and tyrosine in rats used for *in vivo* measurements were affected by the large does of L-phenylalanine which had been injected intravenously. However, even after subtracting the concentrations of L-phenylalanine and tyrosine, the total amino acid concentration *in vivo* appeared to be 40% greater than

TABLE IV

Amino acid concentrations in pancreatic tissue in vivo and following perfusion of the isolated pancreas in vitro

Results are mean ± S.E. of seven pancreata *in vivo* and three pancreata *in vitro* (perfused for a total period of 50 min). [a] Total amino acid concentration excludes phenylalanine and its metabolite tyrosine since a large dose of L-phenylalanine was injected during these experiments; n.d., not detectable.

Amino acid	Concn. (μmol/g pancreas wet wt.)		
	in vivo	perfused pancreas	
		+ amino acids + CCh	Amino-acid-free, CCh-free
Essentials			
Arginine	0.43 ± 0.03	0.14 ± 0.01	0.04 ± 0.01
Isoleucine	0.32 ± 0.08	0.11 ± 0.01	0.03 ± 0.01
Leucine	0.20 ± 0.04	0.15 ± 0.01	0.03 ± 0.01
Lysine	0.55 ± 0.10	0.23 ± 0.02	n.d.
Methionine	0.14 ± 0.01	n.d.	n.d.
Phenylalanine	4.10 ± 0.26	0.14 ± 0.02	0.18 ± 0.02
Threonine	0.80 ± 0.12	0.52 ± 0.09	0.23 ± 0.08
Valine	0.35 ± 0.07	0.37 ± 0.09	0.05 ± 0.01
Non-essential			
Alanine	0.55 ± 0.05	0.84 ± 0.10	0.16 ± 0.01
Asparagine	n.d.	0.06 ± 0.01	n.d.
Aspartate	1.17 ± 0.05	0.94 ± 0.15	0.31 ± 0.03
Glutamate	6.75 ± 0.52	2.41 ± 0.19	1.11 ± 0.09
Glutamine	1.95 ± 0.25	0.48 ± 0.02	0.50 ± 0.06
Glycine	2.47 ± 0.16	1.35 ± 0.12	0.93 ± 0.09
Serine	0.62 ± 0.08	0.43 ± 0.06	0.17 ± 0.01
Tyrosine	1.32 ± 0.31	0.10 ± 0.02	n.d.
Total	15.85 [a]	8.31	3.77

that in pancreata perfused with plasma concentrations of amino acids.

Discussion

The exocrine pancreas avidly accumulates amino acids against a large concentration gradient [19,25,26] and exhibits high protein synthetic activity [8,11–13]. Previous studies from our laboratory have shown that the influx of L-phenylalanine into the exocrine epithelium of the isolated perfused rat pancreas is mediated predominantly via a large neutral Na^+-independent system L [24]. Though the isolated perfused pancreas has been used extensively for physiological and transport studies, rates of protein synthesis have not, to our knowledge, been reported in this model. It has been suggested that the transport of substrates into the pancreatic epithelium may be a limiting factor for the high protein synthetic rates observed in this organ. This study establishes that the rate of amino acid influx across the pancreatic exocrine epithelium is considerably higher than the rate of protein synthesis measured *in vivo* or *in vitro*, indicating that transport was not rate-limiting for protein synthesis.

Effects of amino acids and carbachol on protein synthesis and L-phenylalanine influx

Contradictory observations have been made regarding the immediate source (intracellular or extracellular) of amino acids for incorporation into pancreatic protein [8,26,27]. In a detailed investigation using rat pancreatic fragments, van Venrooij et al. [27] concluded that the amino acids incorporated into protein are derived directly from the extracellular pool. This would imply that the charging of t-RNA is linked directly to membrane transport (see review, Ref. 8). However, the intracellular pool may make a significant contribution to protein synthesis when extracellular amino acid supply is reduced [28].

In the present study the rate of protein synthesis measured in the perfused pancreas was responsive to added amino acids. Even without the addition of these substrates to the perfusion medium, amino acids were still present in pancreatic tissue, presumably as a result of protein degradation, which allowed continued incorporation into protein, though at a reduced rate compared with the presence of amino acids (Table I). Under these conditions amino acid content of the perfused pancreas was reduced by half as a result of a reduction in the concentration of most individual amino acids with the exception of L-phenylalanine, which was present in the perfusate (Table IV). Since these measurements were performed at the end of a 30 min perfusion period, and since the rate of protein synthesis was not significantly different when measured at 5, 15 or 30 min following a pre-equilibration period of 20 min, it could be argued that in the absence of a source of extracellular amino acids protein synthesis proceeds at a constant but reduced rate, possibly determined by the availability of certain intracellular amino acids. However, although intracellular amino acids clearly can act as substrates for protein synthesis under these conditions, it is still possible that extracellular amino acids are the major precursors for protein synthesis *in vivo* under normal conditions.

Carbachol and other pancreatic secretagogues are known to influence pancreatic protein synthesis, but their effects appear to be biphasic in relation to agonist concentration [6,7,9,29]. For example, in fragments of rat pancreas the incorporation into protein of labelled leucine, methionine and tyrosine was reduced by high concentrations of carbamylcholine ($> 10^{-6}$ M), pancreozymin (> 0.1 unit/ml) and caerulein (> 2 ng/ml) [29]. In contrast, in isolated pancreatic acini a low dose of CCK_8 (10^{-10} M) markedly stimulated the rate of incorporation of L-[^{3}H]phenylalanine [9] and L-[^{3}H]leucine [6] into pancreatic protein. Similarly, following *in vivo* infusion of maximal secretory doses of caerulein, L-[^{3}H]leucine incorporation into protein *In vitro* was enhanced [7]. Carbachol at maximal stimulatory concentration ($3 \cdot 10^{-6}$ M) caused a 44% increase

in L-[^{3}H]leucine incorporation into protein [6] but exhibited a maximal inhibitory effect at 10^{-4} M [6]. In the present study $3 \cdot 10^{-7}$ M carbachol stimulated protein synthesis by about 30% (Table III). However, this effect of the secretagogue was only observed when pancreata were perfused with added amino acids. The mechanism responsible for this increase in protein synthesis is presently unknown, though we can exclude a stimulatory effect on L-phenylalanine influx into the cell (see Table I).

Time-dependent changes in amino acid incorporation into protein have been observed in response to injection of cholecystokinin-pancreozymin [30]. In this study the rate of ^{14}C incorporation into total pancreatic protein following an intraperitoneal injection of a tracer dose of L-[^{14}C]phenylalanine was decreased after 5 min, maximally stimulated by 17 min and returned to control value after 45 min [30]. In our preparations carbachol stimulated total protein synthesis at both 5 and 30 min in the perfused pancreas and caused a marked increase in enzyme secretion over the whole 30 min period.

The little information available on the effects of secretagogues on amino acid transport in the pancreas suggests both stimulatory and inhibitory effects – depending on agonist concentration – on the accumulation of α-aminoisobutyric acid (AIB), a nonmetabolizable amino acid analogue [7]. Support for the inhibition of L-phenylalanine influx by carbachol in the absence of amino acids may be found in previous studies. In mouse pancreatic acini not only was AIB uptake in the absence of amino acids inhibited by carbachol (and other secretagogues), but efflux was also accelerated from the preloaded tissue [31]. Studies from our laboratory [32] have shown that carbachol ($3 \cdot 10^{-7}$ M) abolished net α-methylaminoisobutyric acid (MeAIB) uptake via the Na^+-dependent System A [24] whilst stimulating MeAIB efflux. In the pancreas the wide tolerance of System A for different amino acids [24], including L-phenylalanine, suggests that the reduction in L-phenylalanine uptake may reflect decreased uptake via the secretagogue-sensitive System A.

Protein synthesis in relation to influx of L-phenylalanine

The protein synthetic rates measured in vivo in overnight fasted animals (118% day^{-1}) compared well with other studies in rat pancreas. Sakamoto et al. [11] reported rates of 142–293% day^{-1} in animals of similar age using a 6 h constant infusion of labelled tyrosine, a method which is known to yield less reliable results than the flooding dose technique which we employed, particularly when applied to tissues with a high rate of protein synthesis [22,23]. Permutt et al. [12] reported rates of 185% day^{-1} in much younger (4-week-old) non-fasted rats using a flooding dose technique similar to that used here. It is likely that overnight fasting causes a reduction in protein synthesis in the pancreas, as it does in many other tissues [33]. In our study the *in vivo* protein synthetic rate was about 5-fold greater than that measured *in vitro*. Nevertheless, the perfused pancreas exhibited not only an ability to synthesise and secrete proteins but was also responsive to the addition of amino acids and carbachol. The fact that the perfused pancreas had a significantly lower free amino acid content – even with the addition of amino acids in the perfusate – than the pancreas *in vivo* may at least partially explain the difference in the protein synthetic rates between *in vivo* and *in vitro* situations.

Even the highest rate of protein synthesis recorded in the literature, 293% day^{-1} [11], corresponding to about 90 nmol/min per g, is considerably less than the unidirectional influx (121 nmol/min per g) of L-phenylalanine (Table I). Thus, we have demonstrated that pancreatic protein synthesis *in vivo* or in the pancreas perfused *in vitro* under basal or secretagogue-stimulated conditions, would not be limited by amino acid transport into the epithelium.

Acknowledgements

This work was supported by a research grant from ONO Pharmaceutical Co., Osaka, Japan (G.E.M., J.H.S.) M.M. was the recipient of a British Council Fleming Award.

References

1 Morisset, J.A. and Webster, P.D. (1972) J. Clin. Invest. 51, 1–8.
2 Dagorn, J.C. and Lahaie, R.G. (1981) Biochim. Biophys. Acta 654, 111–118.
3 Vieira-Matos, A.N. and Tenenhouse, A. (1977) Can. J. Physiol. Pharmacol. 55, 90–97.
4 Prost, J., Belleville, J. and Valantin-Rollet, C. (1990) Nutrition 6, 247–253.
5 Korsten, M.A., Wilson, J.S. and Lieber, C.S. (1990) Gastroenterology 99, 229–236.
6 Kore, M., Bailey, A.C. and Williams, J.A. (1981) Am. J. Physiol. 241, G116–G121.
7 Adler, G., Bieger, W. and Kern, H.F. (1978) Cell Tissue Res. 194, 447–462.
8 Case, M. (1978) Biol. Rev. 53, 211–354.
9 Lahaie, R.G. (1986) Pancreas 1, 403–410.
10 Rausch, U., Rüdiger, K., Vasiloudes, P., Kern, H. and Scheele, G. (1986) Pancreas 1, 522–528.
11 Sakamoto, A., Moldawer, L.L., Palombo, D.J., Desai, S.P., Bistrian, B.R. and Blackburn, G.L. (1983) Clin. Sci 64, 321–331.
12 Permutt, M.A., Kakita, K., Malinas, P., Karl, I., Bonner-Weir, S., Weir, G. and Giddings, S.J. (1984) J. Clin. Invest. 73, 1344–1350.
13 Attaix, D., Aurousseau, E., Bayle, G., Rosolowska-Huszcz, D., Manghebati, G. and Arnal, M. (1989) J. Nutr. 119, 463–470.
14 Schick, J., Verspohl, R., Kern, H. and Scheele, G. (1984) Am. J. Physiol. 247, G611–G616.
15 Mann, G.E., Muñoz, M. and Peran, S. (1986) Biochim. Biophys. Acta 862, 119–126.

16 Muñoz, M., Emery, P.W., Peran, S. and Mann, G.E. (1988) Biochim. Biophys. Acta 945, 273–280.
17 Emery, P.W., Mann, G.E., Muñoz, M. and Sweiry, J.H. (1990) J. Physiol. 423, 53P.
18 Kanno, T., Suga, T. and Yamamoto, M. (1976) Jpn. J. Physiol. 34, 167–180.
19 Mann, G.E. Smith, S.A., Norman, P.S.R. and Emery, P.W. (1988) Pancreas 3, 67–76.
20 Mann, G.E., Norman, P.S.R. and Smith, I.C.H. (1989) J. Physiol. 416, 485–502.
21 Yudilevich, D.L., and Mann, G.E. (1982) Fed. Proc. 41, 3045–3053.
22 Garlick, P.J., McNurlan, M.A. and Preedy, V.r. (1980) Biochem. J. 192, 719–723.
23 Suzuki, O. and Yagi, K. (1976) Anal. Biochem. 75, 192–200.
24 Mann, G.E. and Peran, S. (1986) Biochim. Biophys. Acta 858, 263–274.
25 Clayman, S. and Scholefield, P.G. (1969) Biochim. Biophys. Acta 173, 277–289.
26 Bieger, W., Peter, S. and Kern, H.F. (1977) Cell Tissue Res. 180, 31–44.
27 Van Venrooij, W.J., Poort, C., Kramer, M.F. and Jansen, M.T. (1972) Eur. J. Immunol. 30, 427–433.
28 Ward, L.C., Buttery, P.J. and Boorman, K.N. (1984) Int. J. Biochem. 16, 1077–1081.
29 Bauduin, H., Tondeur, T., Van Sande, J. and Vincent, D. (1973) Biochim. Biophys. Acta 304, 81–92.
30 Mongeau, R., Dagorn, J.C. and Morissett, J. (1976) Can. J. Physiol. Pharmacol. 54, 305–313.
31 Iwamoto, Y. and Williams, J.A. (1980) Am. J. Physiol. 238, G440–G444.
32 Norman, P.S.R. and Mann, G.E. (1988) Biochim. Biophys. Acta, 943, 541–546.
33 Waterlow, J.C. (1984) Q.J. Exp. Physiol. 69, 409–438.