Paradoxical effects of endogenous and exogenous insulin on amino acid transport activity in the isolated rat pancreas: somatostatin-14 inhibits insulin action

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Summary. Regulatory effects of insulin, somatostatin and cholecystokinin on amino acid transport in the isolated perfused rat pancreas have been studied using a rapid dual isotope dilution technique. Uni-directional L-serine transport (15 s) was quantified relative to an extracellular tracer D-mannitol over a wide range of substrate concentrations. In pancreata perfused with 2.5 mmol/l D-glucose, a weighted non-linear regression analysis of overall transport indicated an apparent $K_m = 14.4 \pm 1.6$ mmol/l and $V_{max} = 25.9 \pm 1.4$ $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ($n=6$). Although L-serine transport was stimulated during perfusion with 100 $\mu\text{U/ml}$ bovine insulin, endogenous insulin ($7\text{--}25$ ng $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$) released during continuous perfusion with either 8.8 mmol/l or 16.8 mmol/l D-glucose had no such effect. Exogenous somatostatin-14 (250 pg/ml) or cholecystokinin octapeptide (CCK-8, 3×10^{-11} mol/l) appeared to increase only the K_m for transport.

Only CCK-8 evoked a notable protein output (2.9 ± 0.3 mg $\cdot 30 \text{ min}^{-1} \cdot \text{g}^{-1}$) and juice flow (68 ± 10 $\mu\text{l} \cdot 30 \text{ min}^{-1} \cdot \text{g}^{-1}$, $n=3$) from the exocrine pancreas. When pancreata were perfused with bovine insulin (100 $\mu\text{U/ml}$) and somatostatin-14 (250 pg/ml), the stimulatory action of exogenous insulin on L-serine transport was abolished. If endogenous insulin and somatostatin, released concurrently in response to 16.8 mmol/l D-glucose, were conveyed to the exocrine epithelium via an islet-acinar portal axis, it is conceivable that somatostatin modulates the stimulatory action of insulin on basolateral amino acid transport in the exocrine pancreas.

Key words: Amino acid transport, insulin, somatostatin, cholecystokinin octapeptide, pancreatic secretion, glucose challenge, exocrine pancreas (rat).

In the rat pancreas, morphological [1] and functional [2, 3] evidence supports the existence of a portal circulation conveying hormones from the islets of Langerhans directly to the basolateral membrane of the exocrine epithelium [4, 5]. The extent of this insulo-acinar portal axis has recently been questioned [6] since rat islets also possess efferent vessels which bypass the exocrine cells and lead directly into the systemic circulation [1, 6]. Although such veins have been identified in 60% of rat islets, most of the efferent vessels from smaller islets within the exocrine lobules appear to be insulo-acinar portal vessels [1].

Receptors for insulin [5] and somatostatin [7–10] have been identified on the basolateral surface on pancreatic acinar cells; however, a direct regulatory action for glucagon remains to be established [11]. Specific receptors for cholecystokinin and other cholinergic secretagogues have also been localised in pancreatic acini [12]. In the isolated perfused rat pancreas, exogenous insulin and endogenous insulin, released during a glucose challenge, potentiate enzyme and juice secretion

evoked by cholecystokinin [2]. Recent studies in the perfused rat pancreas examined the effects of reducing endogenous insulin bioavailability on enzyme release induced by caerulein, a cholecystokinin analog [3]. Pancreatic enzyme secretion was inhibited in the presence of anti-insulin serum and in preparations isolated from insulin deficient streptozotocin-diabetic rats. By contrast, caerulein-induced amylase release was similar in pancreatic acini isolated from normal or diabetic rats, indicating that disruption of the insulo-acinar portal axis removes the influence of basal insulin secretion on the exocrine pancreas [3].

Insulin and streptozotocin-induced diabetes have previously been shown to modulate small, neutral amino acid uptake in the rat pancreas perfused in situ [13]. Stimulation of L-serine transport by bovine insulin was found to be ouabain-insensitive and dependent upon extracellular calcium [14]. Transport was largely mediated by a novel Na^+ -independent system [15] resembling System asc (alanine, serine, cysteine) described in horse erythrocytes [16].

The present study was undertaken to determine whether endogenous insulin, released during perfusion of the rat pancreas with 8.8 or 16.8 mmol/l glucose, could mimic the stimulatory action of exogenous bovine insulin on L-serine transport. As glucose also stimulates release of pancreatic somatostatin [17–22], we investigated the effects of exogenous somatostatin-14 on L-serine transport in the absence and presence of 100 μ U/ml insulin. For comparison, we studied whether cholecystokinin octapeptide (CCK-8), a pancreatic secretagogue known to elevate intracellular Ca^{2+} , could influence pancreatic amino acid transport. Abstracts of this work have been presented [23, 24].

Materials and methods

Animals

Male Sprague-Dawley rats had free access to water and a standard laboratory chow diet (No. 491, Grain Harvesters Ltd, Kent, UK). Prior to the experiment, animals (180–220 g) were fasted for 24 h and then anaesthetised between 09.00 and 13.00 hours with an i.p. injection of sodium pentobarbitone (Sagatal 6 mg/100 g).

Isolation and perfusion of the pancreas

The pancreas was isolated, as described by Kanno et al. [25], and perfused at constant flow (1.75 ml/min) with a Krebs-Henseleit bicarbonate medium via the coeliac and superior mesenteric arteries with the portal vein as outlet. The isolated preparation was placed in a tissue bath maintained in a temperature controlled (38°C) perfusion cabinet [14]. The pancreatic venous effluent was collected from the cannulated portal vein, and flow rate and perfusion pressures were monitored throughout an experiment.

Perfusates

The perfusate had the following composition (mmol/l): 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; Dextran T70 (5% w/v, Pharmacia, Milton Keynes, UK); bovine serum albumin (0.25% w/v, Cohn Fraction V, Sigma Chemical Company, Poole, Dorset, UK). Perfusate free of amino acids and dextran was added to the tissue bath (50 ml).

Different concentrations of L-serine (0.05–50 mmol/l) were added to the vascular perfusates to examine the influence of hormones on the kinetics of amino acid transport. Non-specific effects of increased osmolarity could be eliminated, since similar transport rates were measured for L-serine (at 0.05 mmol/l) during perfusion in the absence ($40 \pm 8 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, $n=6$) and then presence ($36 \pm 6 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) of 50 mmol/l D-mannitol.

Exogenous islet and gastrointestinal hormones

When bovine insulin (100 μ U/ml, Wellcome, Dartford, UK), somatostatin-14 (250 pg/ml, Peninsula Laboratories, Merseyside, UK), or cholecystokinin octapeptide (3×10^{-11} mol/l sulphated CCK-8, Peninsula) were added to the perfusates, pancreata were initially pre-perfused for 20–25 min with the hormone(s). L-serine transport was then measured in the continued presence of hormone(s). Solutions were gassed with 95% O_2 /5% CO_2 at 38°C to a pH between 7.3–7.4.

Measurement of amino acid uptake and efflux in the exocrine pancreas

Amino acid uptake was measured using a rapid dual isotope dilution technique [26] previously applied to the isolated perfused rat pancreas [13–15]. Islets comprise only 2%–4% of the pancreatic weight and rapidly oxidise D-glucose [27], whereas the exocrine pancreas preferentially utilises extracellular amino acids [28] for energy metabolism [27]. Although rat pancreatic islets transport neutral amino acids [29], rates of influx measured during incubation periods of 2 to 15 min appear at least 2-fold lower than our influx values measured over 15 s in the perfused rat pancreas [15].

The basolateral pancreatic epithelium would be expected to be the limiting area for exchange in our transport studies, because our pilot studies have shown that D-mannitol and amino acids freely traverse the highly permeable fenestrated capillaries [30]. We have used light microscopic autoradiography to localise cellular sites of $\text{L-}[^3\text{H}]\text{serine}$ uptake, and detected a lower silver grain density above peri-islet acini compared to islet-distant acini [31]. The absence of silver grains in the vasculature and interstitium together with the well-known protein synthetic capacity of the exocrine pancreas [30] strongly suggests that the initial uptake of L-serine was mediated by a basolateral plasma membrane carrier(s).

Overall uptake of $\text{L-}[^3\text{H}]\text{serine}$ was referred to the dilution profile of an extracellular reference molecule $\text{D-}[^{14}\text{C}]\text{mannitol}$ and simultaneous portal vein tracer concentration-time curves were obtained following a pulsed arterial injection (100 μ l/2 s) of perfusate containing both labelled molecules (Fig. 1A). Twenty to thirty 100 μ l samples were immediately collected sequentially from the portal vein during 45–60 s, and a final sample was accumulated for a further 4 min to maximise tracer recoveries (data not shown). The time course of $\text{L-}[^3\text{H}]\text{serine}$ uptake was quantified from successive venous samples using the equation: fractional uptake = $\{1 - (\text{L-}[^3\text{H}]\text{serine} / \text{D-}[^{14}\text{C}]\text{mannitol})\}$.

Uni-directional $\text{L-}[^3\text{H}]\text{serine}$ uptake (U_{max}), measured at different perfusate concentrations of unlabelled L-serine, was then used to estimate amino acid influx (v): $v = [F \cdot \ln(1 - U_{\text{max}}) \cdot C_a]$, where C_a is the perfusate concentration of L-serine and F the perfusion rate in $\text{ml} \cdot \text{min}^{-1}$ per gram of pancreas wet weight. Pancreata were equilibrated for 4 min with a given L-serine concentration before measuring rapid (15 s) $\text{L-}[^3\text{H}]\text{serine}$ uptake in the continued presence of substrate and absence or presence of hormone(s). Influx values determined at different substrate concentrations were then used to obtain Michaelis-Menten kinetic constants, and a single entry site analysis revealed the lowest weighted standard deviation of residuals.

$\text{L-}[^3\text{H}]\text{serine}$ efflux was estimated from the difference in uni-directional (15 s) and net (5 min) tracer uptakes: $\% \text{efflux} = \{(1 - U_T / U_{\text{max}}) \cdot 100\}$, where U_T is the net uptake determined from the integrated recoveries of $\text{L-}[^3\text{H}]\text{serine}$ and $\text{D-}[^{14}\text{C}]\text{mannitol}$ during a total 5 min venous collection period.

Pancreatic protein output and juice secretion

Pancreatic secretion evoked by CCK-8 was measured using a calibrated silicon tube (0.5 mm external diameter) attached to the free end of the pancreatic duct cannula [2,25]. The tubing was replaced at 10 min intervals; pancreatic juice volumes were diluted with 0.9% NaCl containing phosphate buffer (pH 7.1); and total protein was assayed using the method of Lowry et al. [32], with bovine serum albumin as a standard.

Insulin and glucagon radioimmunoassays

Samples of the pancreatic venous outflow were collected at 1- or 2-min intervals into chilled tubes containing 30 μ l aprotinin (Trasylol, Bayer, Newbury, UK) and frozen at -20°C until the time of assay.

Fig. 1a and b. Analysis of L-serine transport at the basolateral membrane of the rat exocrine pancreatic epithelium. **a** Paired venous dilution profiles obtained for L-[3-³H]serine (○—○) and the extracellular tracer D-[1-¹⁴C]mannitol (●—●) following a rapid pulse injection (100 μl in 1–2 s) of both tracers into the arterial inflow of the isolated perfused pancreas. Recoveries in successive venous samples are expressed as a percentage of the arterially injected radioactive dose and plotted against the venous collection time. **b** Time course of unidirectional L-[3-³H]serine uptake relative to D-[1-¹⁴C]mannitol (■) was determined from the dilution curves in **a**: uptake% = [L-(³H)serine/D-(¹⁴C)mannitol] × 100. The dotted line (---) indicates that L-[3-³H]serine uptake decreased with time due to significant tracer efflux from the epithelium.

Table 1. Transport of L-serine at the basolateral plasma membrane of the pancreatic epithelium

L-serine (mmol/l)	Perfusion pressure (mmHg)	Perfusion flow (ml · min ⁻¹ · g ⁻¹)	% Uni-directional uptake (15 s)	% Net uptake (5 min)	% Efflux
0.05	32 ± 4	1.47 ± 0.07	77 ± 2	37 ± 2	61 ± 3
1	37 ± 5	1.44 ± 0.07	64 ± 4	39 ± 4	59 ± 8
5	35 ± 4	1.41 ± 0.05	59 ± 5	27 ± 4	72 ± 3
10	34 ± 4	1.42 ± 0.06	50 ± 5	16 ± 3	72 ± 4
20	36 ± 6	1.41 ± 0.06	37 ± 5	10 ± 2	78 ± 5
30	35 ± 4	1.39 ± 0.06	34 ± 2	8 ± 1	80 ± 4
50	36 ± 3	1.41 ± 0.06	27 ± 2	6 ± 1	84 ± 5

Pancreata were isolated from 24-h fasted rats and perfused at constant flow with different unlabelled L-serine concentrations. Efflux of L-[³H]serine was estimated from the difference in uni-directional and net tracer amino acid uptake (see Methods). Perfusion parameters are tabulated, and all values denote the mean ± SEM of measurements (one at each concentration) in each of 6 rats.

Duplicate 100 μl aliquots of the venous samples were assayed and release was quantified as ng/min per gram wet weight of pancreas. The tracers were [¹²⁵I]rat insulin and [¹²⁵I]pig/bovine glucagon, and standards were purified rat insulin (Lot R170, Novo, Bagsvaerd, Denmark) and pig/bovine glucagon (Novo). Details have previously been published [33]; the limit of detection for these assays was 0.08 ng/ml for insulin and 78 pg/ml for glucagon.

Radioactive molecules and counting procedures

The radioactive molecules L-[3-³H]serine (0.7 TBq/mmol) and D-[1-¹⁴C]mannitol (2 GBq/mmol) were purchased from, respectively, Amersham International plc, Aylesbury, UK and New England Nuclear Chemicals, Dreieich, FRG. Samples and appropriate isotope standards were counted concurrently on a Beckman LS7500 liquid scintillation counter interfaced to a microcomputer.

Statistical analysis

Data are expressed as means ± SEM of measurements in number of animals. Analyses were performed using the two-tailed Student's *t*-test for unpaired data.

Results

Self-inhibition of L-serine transport

Figure 1a shows simultaneous venous dilution profiles obtained for labelled L-serine and D-mannitol (extracellular reference) following a rapid injection of both tracers into the arterial supply of the isolated perfused rat pancreas. The lower recovery of L-[³H]serine

Table 2. Kinetics of uni-directional L-serine transport in the absence and presence of insulin and somatostatin-14

Condition	Number ^a	K _m (mmol/l)	V _{max} (μmol · min ⁻¹ · g ⁻¹)	V _{max} /K _m
Control perfusate	34 (6)	14.4 ± 1.6	25.9 ± 1.4	1.79
+ Insulin (100 μU/ml)	31 (7)	29.8 ± 5.7 ^b	57.1 ± 6.0 ^c	1.92
+ Somatostatin-14 (250 pg/ml)	24 (4)	19.6 ± 1.3 ^b	24.1 ± 0.7	1.23
+ Insulin (100 μU/ml)				
Somatostatin-14 (250 pg/ml)	28 (5)	19.9 ± 1.9 ^b	17.1 ± 0.8 ^c	0.86
+ CCK-8 (3 × 10 ⁻¹¹ mol/l)	21 (3)	21.9 ± 1.5 ^b	29.2 ± 0.96	1.33

^a Values denote the number of observations in number of animals; ^b *p* < 0.05; ^c *p* < 0.007 compared to control K_m and V_{max} values. Bovine insulin, and/or somatostatin-14 or CCK-8 were added to the perfusates and pancreata were pre-perfused for 20–25 min before measuring the kinetics of L-serine influx in the continued absence or presence of the hormone(s). The tabulated kinetic constants were calculated using a weighted Michaelis-Menten analysis.

relative to D-[¹⁴C]mannitol reflects epithelial amino acid uptake, and the time course of uptake is shown in Figure 1b. Uptake reached a maximum 15 s after injection of the tracers and gradually decreased after 60 s due to efflux of tritiated serine from the epithelium. During perfusion of the pancreas with 0.05 mmol/l L-serine, unidirectional tracer uptake (77 ± 2%, *n* = 6) was significantly higher (*p* < 0.001) than net uptake (37 ± 2%) measured over the total 5 min collection peri-

Fig. 2. Self-inhibition of L-[3 H]serine influx in the isolated perfused pancreas. Preparations were continually perfused with 0.05 mmol/l L-serine and amino acid influx was measured in the absence (J_c) or presence (J_i) of increasing concentrations of unlabelled L-serine (1–50 mmol/l). Inhibition of influx was estimated from a decrease in the ratio of J_i/J_c (◆—◆). Values denote mean $\pm$ SEM in 6 rats. I = SEM

Fig. 3. Effects of exogenous insulin and D-glucose on L-serine transport. Pre-equilibrated pancreata were perfused for 20–25 min with 2.5 mmol/l D-glucose in the absence (□—□) or presence of 100 μ U/ml bovine insulin (▲—▲). Other preparations were perfused for 20–25 min with either 8.8 mmol/l (data not shown) or 16.8 mmol/l glucose (■—■). Kinetics of uni-directional L-serine influx (1–50 mmol/l) were measured after this 20–25 min exposure period and during continued perfusion with the above solutions. Michaelis-Menten kinetics were estimated by directly fitting a single weighted rectangular hyperbola to the three sets of mean influx values. Mean influx data were weighted for the reciprocal of their respective standard errors and the vertical lines denote the SEM at each concentration in 4–7 perfused pancreata. $\pm$, $\pm$ = plus or minus SEM

od (Table 1). Even at this low substrate concentration efflux of L-[3 H]serine was $61 \pm 3\%$. Thin-layer chromatographic analysis of the pancreatic effluent confirmed that 5 min after a tracer injection the tritium activity was still associated with native L-serine (data not shown).

Uni-directional and net tracer uptakes were reduced by increasing substrate concentration suggesting cis-inhibition (Table 1). The kinetics of self-inhibition are shown in Figure 2, and exposure to 10 mmol/l L-serine

reduced influx to $45 \pm 5\%$ of control values. Increasing the extracellular L-serine also enhanced tracer efflux (Table 1) presumably by accelerating exchange diffusion of unlabelled substrate for transported L-[3 H]serine [34].

Effects of exogenous insulin

L-serine transport in the presence of 2.5 mmol/l D-glucose was saturable (Fig. 3) with an apparent $K_m = 14.4$ mmol/l and $V_{max} = 25.9 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ (Table 2). This V_{max} value is higher than that measured previously in the pancreas perfused in situ with 5.5 mmol/l D-glucose and 1% bovine albumin [13]. In other experiments, pancreata were perfused for 20–25 min with 2.5 mmol/l glucose and 100 μ U/ml bovine insulin. Thereafter, the kinetics of L-serine transport were measured during a further 40–60 min perfusion with 2.5 mmol/l glucose and 100 μ U/ml bovine insulin. Under these conditions, L-serine transport capacity ($V_{max} = 57.1 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was increased; whereas the transport affinity ($K_m = 29.8$ mmol/l) was lowered (Table 2). Exogenous insulin had no effect on perfusion pressure or flow rate (data not shown). Islet hormone release was not monitored in experiments with insulin.

Effects of endogenous insulin released by a vascular D-glucose challenge

We then investigated whether release of endogenous insulin could mimic the stimulatory effect of bovine insulin on L-serine transport. After a pre-equilibration period with 2.5 mmol/l glucose, pancreata were per-

fused for 20–25 min with 2.5, 8.8 or 16.8 mmol/l glucose. Perfusion with each glucose concentration was then continued for another 40–60 min, and kinetic experiments were only performed after 20–25 min exposure to each glucose concentration. This experimental design mimicks the pre-perfusion period with bovine insulin.

Figure 4 illustrates the effects of challenging pre-equilibrated pancreata (2.5 mmol/l glucose) with either 8.8 or 16.8 mmol/l glucose. The latter glucose concentration evoked the characteristic and well-documented biphasic release of immunoreactive insulin (IRI) and, as previously reported [14], inhibited immunoreactive glucagon (IRG) release. IRI levels in the pancreatic venous effluent were maintained above $20 \text{ ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ (approx 275–300 $\mu\text{U}/\text{ml}$) for over 40 min, confirming earlier reports of long term glucose stimulation of insulin release from the perfused rat pancreas [35]. IRI levels were maintained between $5\text{--}12 \text{ ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ during 60 min perfusion with 8.8 mmol/l glucose (Fig. 4). These latter portal vein levels of endogenous insulin are marginally higher than the $6\text{--}7 \text{ ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ achieved during infusion of $100 \mu\text{U}/\text{ml}$ bovine insulin.

Surprisingly, kinetics of L-serine transport were similar in the presence of 2.5, 8.8 (data not shown) or 16.8 mmol/l glucose (Fig. 3). L-serine influx ($10 \text{ mmol}/\text{l}$) during continued perfusion with 2.5 or 8.8 mmol/l glucose was respectively, $12.9 \pm 1.1 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ and $13.2 \pm 0.6 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ($n=3$).

Release of IRI and IRG was examined in more detail at different glucose and L-serine concentrations. Table 3 summarises these findings, and at 2.5 mmol/l glucose insulin release was low compared to an elevated release of glucagon. Subsequent perfusion with $10 \text{ mmol}/\text{l}$ L-serine had no effect on IRI release but enhanced IRG release. This amino acid-induced stimulation of IRG release was transient and values returned to control levels after 8 min exposure to $10 \text{ mmol}/\text{l}$ L-serine [14]. In the presence of 8.8 mmol/l glucose, IRI release increased and IRG release decreased. Under these conditions, $10 \text{ mmol} \cdot \text{l}^{-1}$ L-serine further enhanced IRI release and inhibited IRG release. IRI release was markedly stimulated by 16.8 mmol/l glucose and L-serine, whereas IRG release remained essentially unaltered except in the presence of $50 \text{ mmol}/\text{l}$ L-serine.

Inhibition of insulin action by exogenous somatostatin-14

Previous studies in the isolated rat pancreas have shown that somatostatin is released with insulin during a vascular D-glucose challenge [17–22]. We have also noted an increase in immunoreactive somatostatin (S14) release from $<10 \text{ pg}/\text{ml}$ to more than $50 \text{ pg}/\text{ml}$ during perfusion of the rat pancreas with 8.8 mmol/l glucose. As in the case of bovine insulin, transport kinetics were

Fig. 4. Immunoreactive insulin release during a prolonged D-glucose challenge of the isolated perfused rat pancreas. Pancreata were perfused with 2.5 mmol/l glucose for 20–25 min and basal IRI release was then monitored for 5 min. The perfusate was then switched to one containing either 8.8 (○—○) or 16.8 mmol/l (●—●) glucose and IRI release was monitored for another 40–60 min. Values denote the mean $\pm$ SEM of duplicate measurements obtained in 3–4 rats. $\pm$ = plus or minus SEM

Table 3. Synergistic effects of D-glucose and serine on immunoreactive insulin and glucagon release from the isolated perfused rat pancreas.

Perfusate D-glucose (mmol/l)	Perfusate L-serine (mmol/l)	Insulin ($\text{ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)	Glucagon ($\text{ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)
2.5		0.4 ± 0.3 (15)	2.0 ± 0.4 (14)
2.5	10	0.14 ± 0.04 (15)	6.3 ± 1.5 (4)
8.8		4.0 ± 0.7 (3)	0.7 ± 0.4 (3)
8.8	10	7.1 ± 0.7 (3)	0.3 ± 0.03 (3)
16.8		27.0 ± 3.1 (4)	0.3 ± 0.05 (4)
16.8	10	56.0 ± 5.3 (4)	0.7 ± 0.1 (4)
16.8	50	58.2 ± 3.6 (4)	3.4 ± 0.6 (3)

Pre-equilibrated pancreata (2.5 mmol/l glucose) were perfused for 20–25 min with 2.5, 8.8 or 16.8 mmol/l D-glucose. Perfusion with each glucose concentration was continued for another 40–60 min in the absence or presence of L-serine (10 and/or 50 mmol/l). Pancreatic effluent samples were collected at 1–2 min intervals for 5 min after switching to a given perfusate. Immunoreactive insulin and glucagon release was expressed as ng/min per gram of pancreas wet weight and averaged over 5-min collection periods. Values denote the mean $\pm$ SEM of duplicate measurements in 3–15 rats

examined during continuous perfusion with exogenous somatostatin-14 (250 pg/ml). This concentration is near the maximal level detected in the perfused rat pancreas [20]. Although the K_m (19.6 mmol/l) for transport increased, there was no significant change in the transport rate (Table 2).

When preparations were perfused with exogenous bovine insulin ($100 \mu\text{U}/\text{ml}$) and somatostatin-14 (250 pg/ml), the previously observed stimulation of L-serine transport by insulin (Fig. 3) was fully inhibited.

Fig. 5a and b. Pancreatic protein output and juice flow in the exocrine pancreas. Dose response curves for total protein output (**a**) and juice flow (**b**) during perfusion of the isolated pancreas with increasing doses of cholecystokinin octapeptide (CCK-8). Values denote the mean $\pm$ SEM of measurements in 3 rats or duplicate determinations in one rat. **a** ● = protein output in $\text{mg} \cdot 30 \text{ min}^{-1} \cdot \text{g}^{-1}$; **b** ● = juice flow in $\mu\text{l} \cdot 30 \text{ min}^{-1} \cdot \text{g}^{-1}$; I = $\pm$ SEM

Fig. 6a and b. Time course of changes in protein output and juice flow during vascular infusion of $3 \times 10^{-11} \text{ mol/l}$ CCK-8 (hatched bars = $\square$). **a** Total protein output was monitored over a 50 min perfusion interval and is expressed as $\mu\text{g}/\text{min}$ per gram pancreas wet weight. **b** Juice flow is expressed as $\mu\text{l}/\text{min}$ per gram pancreas wet weight. Values denote the mean $\pm$ SEM of measurements in 3 rats. I = $\pm$ SEM

The K_m for transport was not different from that observed with somatostatin-14 alone, whereas $V_{\max}$ was reduced below control rates (Table 2). Moreover, the transport index $V_{\max}/K_m$ was decreased from a control value of 1.79 to 0.86.

Effects of exogenous cholecystokinin octapeptide

For comparison, we examined whether the pancreatic secretagogue CCK-8 – known to bind to specific acinar cell receptors – stimulated L-serine transport. Dose response curves for CCK-8 evoked pancreatic protein output and juice flow are shown in Figure 5. The time course of changes in total protein and juice secretion evoked by $3 \times 10^{-11} \text{ mol/l}$ CCK-8 are shown in

Figure 6. Unlike with exogenous insulin, L-serine transport was largely unaffected by CCK-8 (Table 2). The reported inhibitory effect of CCK-8 on somatostatin binding in pancreatic acini [7–9] and the fact that CCK-8 stimulates release of insulin, glucagon and somatostatin from the islets of Langerhans [22, 35, 36] dissuaded us from studying interactions between CCK-8 and other exogenous hormones.

Discussion

In the present study, pancreatic amino acid transport was stimulated by exogenous bovine insulin but paradoxically unaffected by insulin released from the endocrine pancreas during a prolonged vascular D-glucose challenge. Although perfusion with somatostatin-14 or CCK-8 alone only marginally altered the kinetics of L-serine transport, the stimulatory action of exogenous insulin was abolished in the presence of exogenous somatostatin.

Immunoreactive insulin levels ranged from <1 to $25 \text{ ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ during perfusion of the pancreas with 2.5, 8.8 and 16.8 mmol/l glucose. Although supra-maximal hormone levels are often inhibitory [3, 5, 12], such insulin levels have been detected in the effluent of the rat pancreas during a prolonged vascular glucose challenge [37]. In our work 8.8 mmol/l glucose evoked an insulin release in the range achieved during perfusion with bovine insulin, and yet amino acid transport was not stimulated.

Functional evidence supports the existence of a pancreatic insulo-acinar portal circulation [2, 3]. Exogenous rat insulin ($1\text{--}20 \mu\text{U/ml}$) and endogenous

insulin (232 μ U/ml) released during perfusion with 17.5 mmol/l D-glucose potentiated CCK evoked exocrine secretion [2]. Since a non-recirculating perfusion system was used in this study, it appears that insulin modulates exocrine function without entering the systemic circulation [2, 5]. Elevated glucose may have affected glucagon and somatostatin release from the endocrine pancreas [2]. Recent studies in the isolated pancreas have confirmed that glucose stimulates insulin and somatostatin release but inhibits glucagon release from the endocrine pancreas [17–22]. On going experiments in our laboratory revealed that human glucagon (10^{-9} mol/l) has no effect on L-serine transport (data not shown).

Somatostatin-14 is known to bind to specific acinar cell membrane receptors [7, 10] by a calcium-dependent mechanism [8, 9]. It is interesting that the stimulatory action of insulin on pancreatic L-serine transport is abolished upon removal of extracellular calcium [14]. In cultured rat myoblasts, somatostatin reduces insulin-stimulated leucine and aminoisobutyric acid (2-AIB) uptake but has no effect on basal uptake of these substrates, suggesting that circulating insulin and somatostatin may have antagonistic effects on nutrient uptake into cells [38]. In the pancreas, somatostatin similarly reduced insulin-stimulated L-serine transport but had little effect on basal uptake (Table 2). Somatostatin-14 binding sites have been identified on A and B cells in cultured rat islets [39], and low doses of exogenous somatostatin may increase glucagon and insulin release by suppressing endogenous somatostatin release, whereas higher doses mask this effect by directly inhibiting A- and B-cell function [40].

Activation of protein kinase C by phorbol esters (TPA) or diacylglycerol reduces somatostatin binding in pancreatic acini, but the time course of inhibition is longer than that observed for inhibition of insulin binding [41]. The cellular mechanisms by which somatostatin inhibits insulin action in the exocrine pancreas merit further study. Perhaps protein kinase C may be one of the factors modulating islet hormone interactions at the basolateral plasma membrane of the exocrine pancreatic epithelium.

In summary, pancreatic L-serine transport mediated by the Na^+ -independent System asc is sensitive to interactions between insulin and somatostatin. Characteristics and kinetics of this transporter differ from the classical Na^+ -dependent System A mediating L-alanine uptake in pancreatic acini [42]. Lack of an effect with endogenous insulin may reflect efferent vessels leading from the islets directly into the systemic circulation [1, 6]; however, our results are consistent with another hypothesis. If somatostatin were released together with insulin during a vascular D-glucose challenge [17–22] and conveyed to the exocrine pancreas via insulo-acinar portal vessels [1], it is possible that somatostatin regulates the stimulatory actions of insulin on exocrine pancreatic amino acid transport.

Acknowledgements. This study was supported by project grants from the Medical Research Council, UK (GEM). The Wellcome Trust (UK) is gratefully acknowledged for their support of a Summer Vacation Scholarship for Ms R. Kramer. We thank Dr CST Hii and Professor SL Howell for quantifying IRI and IRG release, and Dr JAH Wass and Ms C. Davidson for measuring immunoreactive somatostatin release.

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Received: 10 June 1988
and in revised form: 12 January 1989

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