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Significance of γ-glutamyltranspeptidase in exocrine pancreatic amino acid transport

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The exocrine pancreas is rich in γ-glutamyltranspeptidase (GGT, EC 2.3.2.2) and exhibits high rates of amino acid transport and protein synthesis. The role of the γ-glutamyl cycle in mediating neutral amino acid transport in the isolated perfused rat pancreas was investigated using acivicin, an inhibitor of GGT, and a rapid dual isotope dilution technique. When treatment *in vivo* with acivicin (50 mg/kg) was followed 1 h later by continuous perfusion of the isolated pancreas with 10 μM acivicin, GGT levels decreased from 53 ± 3 IU/g to 4.9 ± 1.5 IU/g. This marked inhibition of GGT activity was not associated with decreased uptake for either L-alanine or L-glutamine, suggesting that the γ-glutamyl cycle plays a negligible role in amino acid transport across the basolateral membrane of the pancreatic epithelium.

Introduction

γ-Glutamyltranspeptidase (GGT, EC 2.3.2.2) is a membrane-bound enzyme which catalyzes transfer of the γ-glutamyl moiety of glutathione (GSH) to a variety of acceptors, including certain amino acids, dipeptides, water or GSH itself [1]. In 1970, Orlowski and Meister [2] postulated that transport of amino acids into cells may be mediated by the γ-glutamyl cycle. According to this hypothesis, γ-glutamyl amino acids are formed in or on the cell membrane by GGT and then transported into cells where the free amino acids are released. However, evidence for an involvement of this cycle in amino acid transport remains controversial. In rat renal brush-border membrane vesicles [3] or pancreatic islets [4] inhibition of GGT activity is not associated with decreased amino acid uptake. In contrast, the activity of GGT appears to be correlated with amino acid transport in lactating mammary gland [5], placenta [6], cerebral endothelial cells [7] and keratinocytes [8].

Among mammalian tissues,the exocrine pancreas has the second highest GGT activity [9] and the pancreatic and kidney enzymes have structural, enzymological and immunological similarities [10]. The physiological relevance of such elevated GGT activity in the pancreas may be related to the high rates of pancreatic amino acid transport [11–16] and protein synthesis (see review Ref. 17). It is therefore of relevance to determine whether the γ-glutamyl cycle plays a role in mediating pancreatic amino acid transport since this cycle could provide a means of regulating transmembrane movement of these substrates. This present study was directed at examining the effects of inhibition of GGT with acivicin (see review Ref. 18) on exocrine pancreatic uptake of L-glutamine and L-alanine, which are both active acceptors of the γ-glutamyl group of GGT [1].

Male Sprague-Dawley rats (200–240 g) had free access to water and a standard laboratory diet (No. 491, Grain Harvesters Ltd, U.K.), or were fasted for either 24 h or 72 h. Pancreata were isolated from anaesthetized rats (60 mg/kg sodium pentobarbitone i.p.), and then perfused *in vitro* as described previously [14–16,19]. Control perfusates had the following composition: NaCl, 131 mM; KCl, 5.6 mM; $CaCl_2$, 2.5 mM; $MgCl_2$, 1.0 mM; NaH_2PO_4, 1.0 mM; $NaHCO_3$, 25 mM; D-glucose, 2.5 mM; bovine serum albumin, 0.25% (w/v) (Cohn Fraction V, Sigma); dextran T70, 5% (w/v) (Meito Sangyo Co., Japan); glutathione, 500 μM; dithiothreitol, 50 μM; and an amino acid mixture (3.11 mM) defined by Allison and Meister [1]. This amino

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TABLE I

Activities of γ-glutamyltranspeptidase in control pancreata and following treatment with acivicin

Results are means ± S.E.; n = number of pancreata.

	γ-Glutamyltranspeptidase (IU/g)
Fed	60.0 ± 7.6 (6)
24h fasted	53.0 ± 3.0 (8)
+ Acivicin (*in vivo*) [a]	3.0 ± 0.9 [d] (4)
+ Acivicin (*in vivo* + *in vitro*) [b]	4.9 ± 1.5 [d] (4)
72h fasted	41.0 ± 3.4 [c] (5)

[a] Acivicin (25 mg/kg) was given I.P. h prior to killing;

[b] Acivicin (50 mg/kg) was given I.P. 1 h before isolation of the pancreas and 10 μM acivicin was added to the perfusion medium (see text for further details).

[c,d] Statistical significance: [c] $P < 0.05$ compared with 24-h fasted group; [d] $P < 0.002$ compared with 24-h fasted.

acid mixture closely approximated concentrations found in rat plasma, thereby providing optimal conditions for transpeptidation by GGT [1]. In some experiments, 24 h fasted rats received a single intraperitoneal injection of acivicin (25 mg/kg or 50 mg/kg) *in vivo*, and 1 h later pancreata were isolated and perfused *in vitro* with 10 μM acivicin for 40–60 min.

Unidirectional amino acid uptake (and efflux) was studied using a dual isotope dilution technique [20], previously applied to the perfused rat pancreas [11–16]. Tracer amino acid uptake was measured by directly comparing portal vein concentration profiles for either L-[2,3-^{3}H]alanine or L-[3,4-^{3}H]glutamine and D-[^{14}C]mannitol (extracellular tracer) following an intra-arterial injection (1–2 s) of a bolus of perfusate containing both tracers. Twenty 100 μl venous samples were collected over 45–60 s and a final venous sample was accumulated for a further 4 min to assess tracer amino acid efflux. The time course of uptake was quantified in successive venous samples from:

$$\text{uptake} = \{1 - (\text{L-}[^3\text{H}]\text{amino acid}/\text{D-}[^{14}\text{C}]\text{mannitol})\}$$

and influx (v) was calculated [11–15] from the maximal fractional tracer uptake (U_{max}), the perfusate amino acid concentration (C_a, mM) and the perfusion rate (F, ml/min per g pancreas wet weight):

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

Backflux (tracer efflux) of a transported amino acid was estimated from:

$$\text{Backflux (\%)} = 1 - U_T / U_{max}$$

where the overall amino acid uptake (U_T) was calculated from the integrated tracer recoveries of the amino acid and D-mannitol over a 5-min interval [12,13,20]. To assay GGT activity in pancreatic homogenates, pancreata were excised, weighed and immediately homogenized with ice-cold Krebs Henseleit bicarbonate buffer as described previously [21]. Data are expressed as means ± S.E. of measurements in n animals.

Pancreatic GGT activity measured in rats fasted for 24 h was not significantly different from the value in fed rats (Table I). In contrast, GGT levels in pancreata isolated from 72-h fasted rats were significantly lower when compared to 24 h starved animals. In order to correlate the present findings with our previous studies of pancreatic amino acid transport [11–16], all subsequent transport studies were performed in pancreata isolated from 24-h fasted rats.

In preliminary experiments pancreata were perfused with 0.25 mM acivicin for 30 min to inhibit GGT activity. Although GGT levels had decreased to 1.8 ±

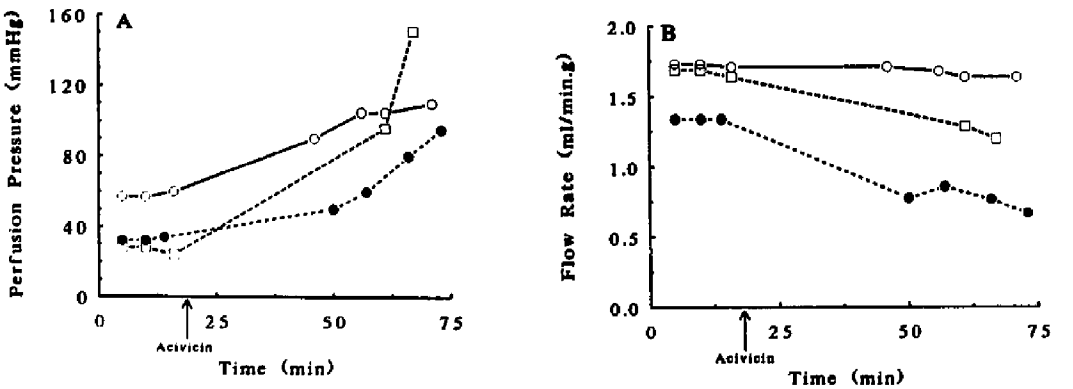

Fig. 1. Effects of acivicin on pancreatic perfusion pressure and flow rate. Isolated pancreata were initially perfused with control media and then continuously with 0.25 mM acivicin (arrow denotes beginning of acivicin infusion) for 50 min. Pancreatic perfusion pressure (A) and flow rate (B) were monitored throughout each of the three experiments shown.

0.2 IU/g ($n = 3$), continuous perfusion with 0.25 mM acivicin resulted in time-dependent increase in pancreatic perfusion pressure and decrease in venous outflow (Fig. 1). In order to avoid the deleterious effects caused by perfusion with high concentrations of acivicin, animals were treated with acivicin (25 mg/kg i.p.) *in vivo* followed 1 h later by isolation and perfusion of the pancreas. After 1 h of acivicin treatment GGT activity decreased to 3 ± 1 IU/g (Table I), however, this inhibition was partially reversed upon perfusion of pancreata with an acivicin-free medium (data not shown). In subsequent experiments, treatment of rats with acivicin (50 mg/kg) *in vivo* followed by perfusion of pancreata 1 h later with 10 μM acivicin resulted in a 90% inhibition of pancreatic GGT activity (Table I). Under these conditions perfusion pressure and flow rate remained stable, and hence this protocol was used to investigate the effects of inhibition of GGT activity on amino acid transport. L-Alanine and L-glutamine were selected as they are amongst the best acceptors of the γ-glutamyl group by GGT [1] and exhibit very high transport rates in the perfused rat pancreas [12,13].

Fig. 2 illustrates uptake profiles for L-[^{3}H]alanine (Panel A) and L-[^{3}H]glutamine (Panel B) in pancreata perfused with acivicin, which are similar to those obtained in the absence of acivicin (data not shown, see Fig. 1 in Ref. 13 and Fig. 1 in Ref. 12, respectively). Despite the marked inhibition of pancreatic GGT activity (Table I), maximum tracer uptake (U_{max}) for L-alanine was higher in acivicin-treated than in control preparations, whereas values for L-glutamine were not significantly different (Table II). Acivicin treatment did not affect net tracer uptake (5-min) or tracer efflux for L-alanine and L-glutamine (Table II). The paradoxical increase in L-alanine and L-glutamine influx observed in acivicin-treated preparations requires further investigation. Since cells apparently accumulate acivicin by a neutral amino acid transporter shared by leucine and glutamine [22], it is conceivable that acivicin may influence influx and efflux of neutral amino acids. Even though acivicin treatment had no inhibitory effects on pancreatic amino acid uptake, we cannot exclude the possibility that cellular accumulation of acivicin may have *trans*-stimulated influx of extracellular alanine or glutamine.

TABLE II

Effects of acivicin on transport parameters measured for L-alanine and L-glutamine in the perfused pancreas

Results are means ± S.E. for four pancreata. See text for definitions of U_{max}, U_T and tracer backflux.

	L-Alanine		L-Glutamine	
	control	acivicin	control	acivicin
U_{max} (%)	39 ± 3	52 ± 4 [b]	47 ± 2	55 ± 5
U_T (%)	8 ± 1	11 ± 1	22 ± 3	25 ± 2
Tracer backflux (%)	93 ± 5	89 ± 2	74 ± 10	65 ± 11
Influx (μmol/min per g)	0.39 ± 0.05	0.73 ± 0.08 [a]	0.44 ± 0.04	0.65 ± 0.07 [b]

[a,b] Statistical analysis (unpaired *t*-tests): [a] $P < 0.001$; [b] $P < 0.05$.

The present findings indicate that GGT plays a negligible role in mediating amino acid uptake across the basolateral membrane of the rat exocrine pancreatic epithelium. The precise localization of GGT in the exocrine pancreas of rodents and man remains controversial [23–26]. Histochemical studies in the rat pancreas have reported an intense GGT activity in the apical portion of acinar cells with only minimal activity in the ductal epithelium and no activity in the islets of Langerhans [23,24]. GGT has also been localized histo-

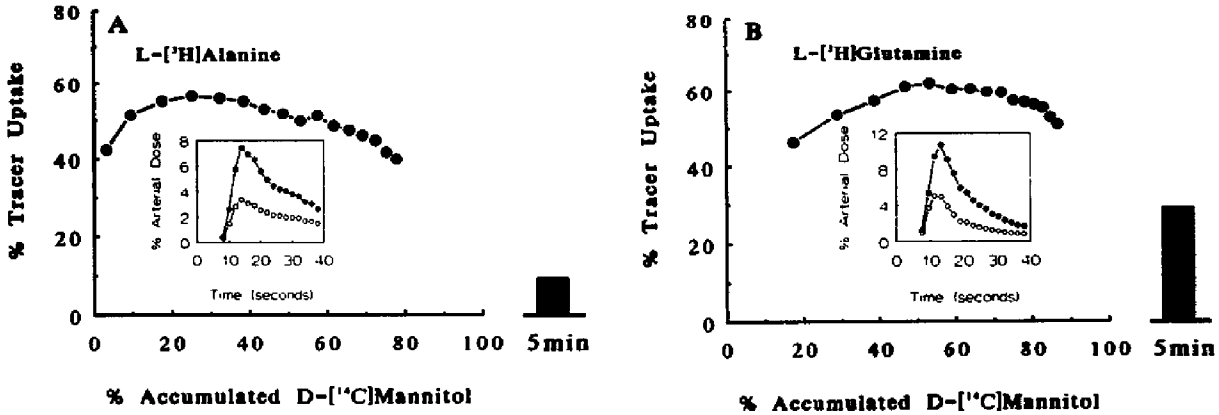

Fig. 2. Pancreatic amino acid uptake in acivicin-treated animals. After *in vivo* treatment with acivicin (50 mg/kg), pancreata were perfused *in vitro* with 10 μM acivicin and uptake of L-[^{3}H]alanine (A) and L-[^{3}H]glutamine (B) was determined. Percentage tracer uptake is plotted against the % accumulated area under the D-[^{14}C]mannitol reference curve. The solid bars denote the net tracer amino acid uptake measured over the 5-min venous sampling interval. Inset in Panel A illustrates venous tracer concentration profiles for D-[^{14}C]mannitol (solid circles) and L-[^{3}H]alanine (open circles) following an intra-arterial injection of a rapid (1–2 s) bolus containing the two isotopes; inset in Panel B shows similar data for L-[^{3}H]glutamine.

chemically in cultured acinar and ductal cells of the hamster pancreas [25]. Recent immunohistochemical studies have concluded that GGT activity is confined predominantly to the luminal surface of centroacinar cells and the ductal epithelium in the human pancreas [26]. It is worth noting that in the human pancreas GGT activity was also localized along the lateral membrane of a limited number of acini in close contact with blood vessels in the connective tissue.

If GGT is primarily localized at the apical surface of pancreatic acinar and ductal cells, then the γ-glutamyl cycle should not necessarily influence amino acid transport at the basolateral membrane of the epithelium. Paracellular pathways in the rat exocrine pancreas are readily permeable to amino acids [27], and hence it is possible that the γ-glutamyl cycle may play a role in the re-uptake of amino acids from pancreatic secretion.

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