γ-GLUTAMYLTRANSPEPTIDASE ACTIVITY IN PANCREATIC ISLETS

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1. Introduction

The enzyme γ-glutamyltranspeptidase catalyzes the transfer of the γ-glutamyl group from a large number of γ-glutamyl compounds including glutathione and glutamine to a wide variety of amino acid acceptors [1]. This enzyme was shown to play a role in amino acid transport [2,3] and in renal NH_4^+ production [4]. This communication indicates that γ-glutamyltranspeptidase activity is present in rat pancreatic islets. The enzyme may participate in the islet metabolism of L-glutamine, reported to represent a physiological fuel for islet cells [5].

2. Materials and methods

Pancreatic islets removed from fed albino rats [6] were homogenized by sonification in a Tris–HCl buffer (100 mM, pH 9.0). The reaction mixture (0.5 ml) consisted of the same Tris–HCl buffer containing, unless otherwise mentioned, $MgCl_2$ (10 mM) and L-γ-glutamyl-*p*-nitroanilide (2.5 mM) [7]. The reaction was initiated by the addition of the islet homogenate (50 μl corresponding to 25–50 islets). After 60 min at 37°C, the reaction was terminated by addition of 0.5 ml 1.5 N acetic acid. The formation of *p*-nitroaniline was determined from the increase in A_{410}, after correction for blank values obtained under identical conditions except that the tissue homogenate was added after the reaction had been terminated.

All results are presented as the mean (±SEM) of individual values, which are each derived from triplicate measurements. The reaction velocity is expressed per μg tissue protein, which was assayed as in [8] using bovine serum albumin as standard. The islet protein content averaged 0.5 ± 0.1 μg/islet (n = 11).

3. Results

The islet homogenate stimulated the liberation of *p*-nitroaniline from γ-glutamyl-*p*-nitroanilide. Over 60 min incubation, the reaction velocity was proportional to the number of islets used, the coefficient of correlation between these variables amounting to 0.993 in the presence of 20, 40 and 60 islets, respectively. The production of *p*-nitroaniline was proportional to the length of incubation. Results collected after 20, 40 or 60 min incubation averaged 0.86 ± 0.11 and 1.57 ± 0.14 nmol . μg protein^{-1} . 60 min^{-1} in the absence and presence of L-glutamine (10 mM), respectively. As already observed with purified kidney γ-glutamyltranspeptidase [9], both monovalent and divalent cations increased the activity of the enzyme in the islet homogenate (table 1). The pH-dependency of enzyme activity was similar in islet and kidney homogenates (fig.1, right).

In a series of 16 expt., the reaction velocity averaged 0.80 ± 0.12 nmol . μg protein^{-1} . 60 min^{-1}. This value represents ~82% of the maximal velocity. In a double reciprocal plot, the reaction velocity at increasing levels of γ-glutamyl-*p*-nitroanilide yielded a straight line with a K_m slightly below 1.0 mM (fig.1, left). The activity of the enzyme in islets was lower (per μg tissue protein) than in kidney and exocrine

Table 1
Effect of different cations upon the reaction velocity of γ-glutamyltranspeptidase in islet homogenates

Na^+ (120–150 mM)	190	Ca^{2+} (1 mM)	148
K^+ (150 mM)	176	Ca^{2+} (10 mM)	193
Li^+ (120–150 mM)	190	Ca^{2+} (50 mM)	224
Choline (120 mM)	166	Mg^{2+} (10 mM)	159

Mean results (n = 1–3) are expressed in % of the paired control value found in the absence of any cation (0.33 ± 0.02 nmol . μg protein^{-1} . 60 min^{-1})

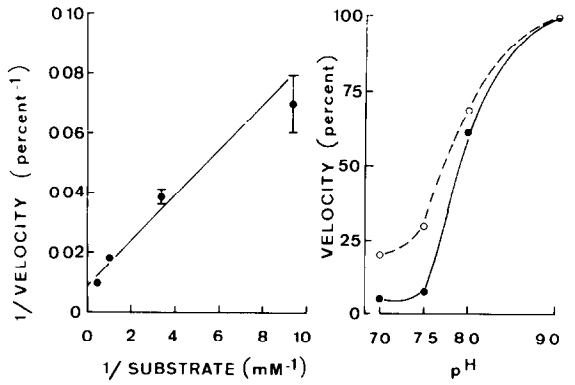

Fig.1. Left: double reciprocal plot for the activity of γ-glutamyltranspeptidase in islet homogenate. Right: effect of pH upon reaction velocity in islet (•, ——) and kidney (○, – – –) homogenates. Reaction velocities are expressed in % of the paired control value found at pH 9.0 in the presence of γ-glutamyl-*p*-nitroanilide: 2.5 mM (right); 3.0 mM (left).

pancreas, but higher than in liver. Thus, relative to the paired value found in kidney (100%), the activity in exocrine pancreas, isolated islets and liver averaged 54.0 ± 5.5, 13.2% ± 5.2% and 2.3% ± 0.8%, respectively ($n = 3$ in each case).

L-Leucine and L-alanine only caused minor and actually insignificant increases in reaction velocity, whereas glycylglycine and, to a lesser extent, L-glutamine significantly augmented the rate of *p*-nitroaniline production (table 2). At a fixed level of γ-glutamyl-*p*-nitroaniline (2.5 mM) and increasing levels of L-glutamine, the reaction velocity increased in proportion ($r = 0.971$) to the concentration of L-glutamine (log scale) over 0.3–10.0 mM, with app. $K_m \sim 1.2$ mM (not shown). There was a highly significant correlation ($r = 0.984$; $p < 0.001$) between the relative ability of different γ-glutamyl acceptors to augment the reaction velocity in islet and kidney homogenates, respectively (table 2). Despite the much lower activity found in liver, there were also significant correlations between the relative effects of various acceptors in liver and either kidney ($r = 0.967$) or islets ($r = 0.935$).

4. Discussion

Our study reveals the presence of γ-glutamyltranspeptidase activity in pancreatic islets. In the islet homogenate, the K_m of the enzyme for γ-glutamyl-*p*-nitroanilide [6] and for the acceptor L-glutamine [1], as well as the influence upon reaction velocity of such factors as pH [1,6], cations [9] and different γ-glutamyl acceptors [1,6] are in good agreement with data collected in other tissues.

The enzyme γ-glutamyltranspeptidase is thought to play a key role in amino acid transport [2,3,10]. This view is compatible with the data collected here in endocrine and exocrine pancreatic tissue, respectively. In the B-cell, the daily output of insulin and C-peptide can be estimated at 7–10% of the cellular total protein content [11]. In the exocrine pancreas, the daily export of protein represents 30–40% of the total protein content [12]. The much higher activity

Table 2
Influence of different γ-glutamyl acceptors upon the activity of γ-glutamyltranspeptidase in islet, kidney and liver homogenates

Acceptor (mM)	Velocity	Islets	Kidney	Liver
Nil	(nmol . μg protein^{-1} . 60 min^{-1}):	0.80 ± 0.12	3.52 ± 0.74	0.06 ± 0.01
Nil		100% (8)	100%	100%
L-glutamate (10)		n.d.	108 ± 3%	85 ± 15%
L-leucine (10)		138 ± 26% (4)	103 ± 3%	94 ± 12%
L-alanine (10)		136 ± 16% (2)	128 ± 2%	140 ± 36%
L-methionine (10)		n.d.	161 ± 3%	141 ± 4%
L-glutamine (10)		184 ± 18%[a] (6)	148 ± 3%	132 ± 7%
L-glutamine (10) + L-leucine (10)		167 ± 15%[b] (4)	143 ± 8%	113 ± 14%
Glycylglycine (10)		315 ± 47%[b] (5)	262 ± 17%	250 ± 29%

Results are expressed either in absolute terms (first line) or in % of the paired control value found in the absence of any acceptor. Mean values (±SEM) for the islet data are shown together with the number of individual determinations (in parentheses) and the significance ([a] $p < 0.01$; [b] $p < 0.02$) of differences from basal value (no acceptor). Kidney and liver data are derived from 2 expt. n.d., not determined

of γ-glutamyltranspeptidase in exocrine as distinct from endocrine pancreas thus coincides with a much higher biosynthetic activity in the acinar tissue.

The presence of γ-glutamyltranspeptidase activity in pancreatic islets may also be relevant to the metabolism of L-glutamine in the endocrine pancreas. We have observed that pancreatic islets convert L-glutamine to glutamate and NH_4^+ at a rate largely exceeding that of glutamate catabolism in mitochondria [5]. A possible explanation for this situation could not be that L-glutamine is converted to glutamate via the γ-glutamyl cycle [10], which in other tissues, represents mainly an extramitochondrial process [4]. The latter hypothesis is compatible with a rather modest glutaminase activity in islet homogenate ($\leqslant$0.5 nmol . islet^{-1} . 60 min^{-1}; A. S., W. J. M., unpublished) relative to the rate of L-glutamine deamidation in intact islets [5].

Acknowledgements

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