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The effect of ethanol on glycyl-prolyl dipeptidyl-aminopeptidase activity in the rat pancreas and liver

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It is well documented that chronic pancreatitis is frequently associated with ethanol abuse. However, the pathogenetic mechanism whereby excessive intake of ethanol results in the injury of pancreas, eventually leading to pancreatic fibrosis, remains yet to be elucidated [1].

Attention has recently been given to the role of X-prolyl dipeptidyl-aminopeptidase, particularly of glycyl-prolyl dipeptidyl-aminopeptidase which is mainly localized in the microsomal fraction of the cell [2], in a series of pathophysiological states concerning collagen metabolism [3, 4]. The present experiment was designed to investigate the change of glycyl-prolyl dipeptidyl-aminopeptidase activity in the rat pancreas and liver under the condition of chronic ethanol feeding with a nutritionally adequate liquid diet.

A total of 17 male Wistar rats, with an average body weight of 214 g at the beginning of the experiment, were divided into two groups. All animals received the liquid diet (Oriental Kobo Co., Tokyo, Japan) according to DeCarli and Lieber [5] for 4 weeks. The diet supplies 18 per cent of the total calories as protein, 35 per cent as lipid, 11 per cent as carbohydrate and 36 per cent either as additional carbohydrate (Control group) or as isocaloric ethanol (Ethanol group).

At the end of the experiment, two pairs of animals were killed by exsanguination from the abdominal aorta after an overnight fast. The pancreas and liver were immediately removed, homogenized in 10 vol. 0.25 M sucrose and centrifuged at 15,000 *g* for 15 min. the supernatant of the homogenate and the serum were taken for enzyme assay. Glycyl-prolyl dipeptidyl-aminopeptidase activity was determined by the method of Nagatsu *et al.* [4], using glycylproline *p*-nitroanilide (Protein Research Foundation, Osaka, Japan) as substrate. Amylase activity was measured with Phadebas amylase test (Pharmacia, Sweden). Both enzymatic activities have been known not to be inhibited in sucrose solution [2, 6].

As expressed in Table 1, amylase activities both in serum and pancreas homogenate were significantly lower in the ethanol group than in the control group. Sardesai and Orten [7] have demonstrated a decrease in protein synthesis by the pancreas after chronic ethanol consumption in rats, although under the short-term administration of ethanol the incorporation of labelled amino acid into various subcellular fractions of the pancreas remained unchanged [8]. The present study revealed that amylase activities in pancreas and serum were significantly decreased after 4 weeks of ethanol feeding, indicating a disturbed synthesis of pancreatic enzyme.

The pancreatic content of glycyl-prolyl dipeptidyl-aminopeptidase, on the other hand, was markedly elevated after ethanol treatment, whereas activity of the enzyme in either serum or liver tissue remained unaffected (Table 1). Figure 1 shows the relationship between amylase and glycyl-prolyl dipeptidyl-aminopeptidase activities in the pancreas. There was a significant ($P < 0.01$) inverse correlation. The rise of pancreatic glycyl-prolyl dipeptidyl-aminopeptidase activity, therefore, seemed to reflect the process linked closely with acinar impairment.

Glycyl-prolyl dipeptidyl-aminopeptidase is regarded as a kind of collagen-peptidase, presumably participating in collagen degradation, since it preferentially liberates dipeptide-fragment glycylproline which is contained abundantly in collagen molecule [4]. A rise of its activity, in fact, has been reported in the experimental granuloma, where collagen turns over rapidly [3]. The findings obtained here,

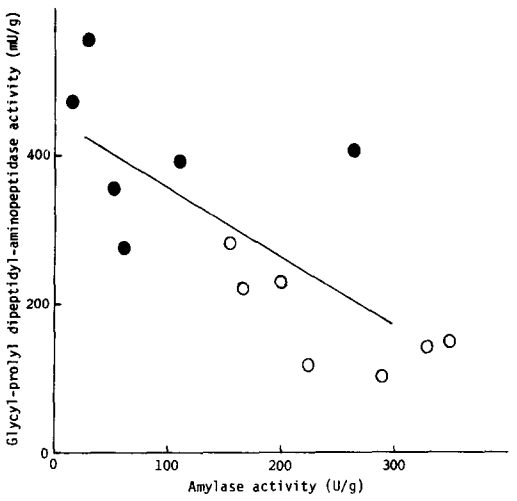

Fig. 1. Relationship between glycyl-prolyl dipeptidyl-aminopeptidase and amylase activities in pancreas. ○: Control; ●: ethanol. $r = -0.73$. $P < 0.01$. $y = -0.92\,x + 447$.

Table 1. Activities of amylase and glycyl-prolyl dipeptidyl-aminopeptidase in serum and homogenates of pancreas and liver in rats

Enzymatic activities	Control group	Ethanol group	Significance*
Amylase			
Serum (U/l)	7443 ± 1195† (10)‡	4474 ± 1295 (6)	$P < 0.001$
Pancreas (U/g tissue)	244 ± 77 (7)	116 ± 103 (7)	$P < 0.05$
Glycyl-prolyl dipeptidyl-aminopeptidase			
Serum (mU/ml)	68.4 ± 12.9 (8)	65.3 ± 19.2 (6)	NS
Pancreas (mU/g tissue)	179 ± 65 (7)	410 ± 96 (6)	$P < 0.001$
Liver (mU/g tissue)	1602 ± 174 (4)	1418 ± 397 (4)	NS

* Significance was measured by Student's *t*-test. NS = not significant.
† Values are expressed as means ± S.D.
‡ Figures in parentheses refer to number of animals.

therefore, may afford some evidence of raised collagen metabolism in the pancreas, but not in the liver, suggesting an initiation of pancreatic fibrosis. The result may also explain the clinical fact that ethanol consumption gives rise to chronic pancreatic lesion within several years, whereas it takes over 10 years for the development of liver cirrhosis [1].

In summary, ethanol administration to rats for 4 weeks caused no change of glycyl-prolyl dipeptidyl-aminopeptidase activity in the liver, but a significant increase in the pancreas, where a concomitant decrease in amylase activity occurred.

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Lipid substitution of mitochondrial monoamine oxidase can lead to the abolition of clorgyline selective inhibition without alteration in the A/B ratio assessed by substrate utilisation

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The mitochondrial monoamine oxidase activity of rat liver can be divided into two categories, MAO A and MAO B, based upon their substrate specificity and the effect of reversible and irreversible inhibitors [2–4]. The model accounting for the rat liver system [2] does not appear to be universally applicable as tissues can vary in the substrate specificity exhibited and their sensitivity to selective inhibitors [3, 4]. Recently MAO A and B have been shown to be different protein species [5–7] with their active sites exposed asymmetrically at the mitochondrial outer membrane [8] and with sialic acid residues playing an important role in the functioning of MAO A[9]. Accepting that MAO A and MAO B may be different protein species there would appear to be a significant heterogeneity within this classification (see [3, 4, 10]). This may result from the binding of membraneous material to the enzymes, a view origiginally proposed by Gorkin and collaborators [11] and for which there is considerable experimental support [3, 12]. Houslay [13] suggested that the selective effects of the irreversible inhibitor clorgyline® (N-methyl-N-propargyl-3-[2,4-dichlorophenoxy] propylamine) may not be directly related to the substrate specificity of the enzyme species but merely resulting from differences in the nature of the environment of the inhibitor binding site. It was proposed [13] that differences in the membrane environment of the propargylamine inhibitor site on MAO A and MAO B accounted for the selective effects of clorgyline®. To test such an hypothesis the, endogeneous lipids of rat liver mitochondrial outer membranes have been replaced by a defined synthetic lipid, dimyristoyl phosphatidyl choline. Such a technique has been successfully used to evaluate the role of the lipid environment in the functioning of other membrane bound enzymes [14, 15].

Rat liver mitochondrial membranes from 200–300 g male Sprague–Dawley rats were purified as described previously [16] with the modification as in [9]. A radiochemical assay