Influence of pyridinolcarbamate (0.3%) on serum and liver cholesterol and cholesterol synthesis in rats (compound fed 3 weeks)

Group[a]	No.	Weight gain (g)	Liver weight (g)	Cholesterol levels		Cholesterol biosynthesis[b]	
				Liver g/100 g	Serum mg/100 ml	Acetate-1-^{14}C (1 μc)	Mevalonate-2-^{14}C (0.5 μc)
Experiment 1							
P	6	42	8.7	370 ± 31[c]	38.9 ± 1.8	0.37 ± 0.02	1.06 ± 0.13
C	6	52	10.0	384 ± 21	33.2 ± 4.3	0.93 ± 0.24	1.14 ± 0.12
Experiment 2							
P	5	106	11.8	364 ± 30	43.2 ± 9.8	0.82 ± 0.20	–
C	5	112	12.4	299 ± 8	47.3 ± 3.6	3.40 ± 0.69	–

[a] P, pyridinolcarbamate; C, control. [b] % conversion. [c] Standard error.

from those of the controls. SHIMAMOTO et al.[2] reported that administration of 10 mg/kg of the test compound to rabbits maintained on 1% cholesterol for 12 weeks did not affect serum cholesterol levels but reduced the cholesterol content of the aorta. The controls and drug-fed rats exhibited no differences in weight gain; the animals in the first experiment weighed 174 ± 3 g at the beginning of the experiment, while those in the second experiment weighed 206 ± 3 g when feeding was begun. Liver weights of the pyridinolcarbamate-fed rats were slightly lower than those of the controls, but the differences were not significant. The liver weight, as % of body weight, was lower in the pyridinolcarbamate-treated rats than in the controls (3.97% vs 4.48% and 3.75% vs 3.93% in experiments 1 and 2, respectively).

Incorporation of acetate-1-^{14}C into cholesterol by rat liver slices was significantly reduced in the pyridinolcarbamate-fed rats ($0.05 > p > 0.02$ in experiment 1 and $0.01 > p > 0.001$ in experiment 2). There was no effect observed in the experiment involving cholesterol biosynthesis from mevalonate-2-^{14}C.

There is no evidence that the effects of pyridinolcarbamate upon human and experimental atherosclerosis[1,2] are mediated through its inhibition of cholesterol synthesis. Nicotinic acid inhibits experimental atherosclerosis in rabbits[7] and has been reported to inhibit cholesterogenesis by some workers[8,9] (but not others[10,11]). However, its hypocholesteremic and anti-atherogenic effect is generally conceded to be due to the inhibition of free fatty acid mobilization[12,13]. The elucidation of the mechanism of action of pyridinolcarbamate must await further investigation[14].

Zusammenfassung. Männliche Wistar-Ratten wurden während 3 Wochen mit 0,3% Pyridinolcarbamat gefüttert, der Cholesterinspiegel in Leber und Serum bestimmt und ausserdem der Einbau von Na-Acetat-1-^{14}C und Mevalonsäure-2-^{14}C im Cholesterin von Leberschnitten gemessen. Der Cholesterinspiegel in Serum und Leber wird durch Pyridinolcarbamat nicht beeinflusst. Der Einbau von Acetat-1-^{14}C in Cholesterin wurde durch Pyridinolcarbamat gehemmt, während die Conversion von Mevalonat-2-^{14}C unbeeinflusst blieb.

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Partial Characterization of Human Pancreatic Carboxypeptidase A

Studies of the chemical and enzymic characterization of bovine[1,2], porcine[3,4] and canine[5] pancreatic carboxypeptidases A (EC 3.4.2.1) have been achieved during the last several years by many workers. However, human pancreatic carboxypeptidase A has never been isolated and characterized to our knowledge. The purpose of this present report is the partial characterization of human pancreatic carboxypeptidase A and the comparison of some of its enzymic properties with those of the bovine and porcine enzymes.

To obtain a purified carboxypeptidase A preparation, 16.9 g of the acetone powder which was prepared from 7 pancreas glands (160 g) by the method of KELLER et al.[6] were extracted with 350 ml of water (dry weight, 10.7 g; $C_1 = 0.022$[7]). The following fractionation with ammonium sulphate was performed with 39% saturation at pH 7.4 (precipitates, 880 mg; $C_1 = 0.049$). For further purification, the precipitates were submitted to chromatography on DEAE-cellulose (linear buffer gradient: 0.005–0.3 *M*

potassium phosphate buffer at pH 8.0). Two active fractions III (36.8 mg; $C_1 = 0{,}16$) and VII (15.8 mg; $C_1 = 0.11$) were obtained. Repeated fractionation of the fraction III under the same column conditions yielded further enriched 2 active fractions (fraction A_1, tube number 99 and 100, 3.66 mg; $C_1 = 1.18$; and fraction A_2, tube number 158 and 159, 0.53 mg; $C_1 = 2.68$).

The pH optimum of these enzyme fractions was 7.5 in both cases when Z-Gly-Phe-OH[8] was used as substrate. The Michaelis constants of A_1 and A_2 for hydrolysis of Z-Gly-Phe-OH were 30.8 mM and 36.6 mM (cf ref. [3], bovine A, 16.6 mM; porcine A_1 and A_2, 16.6 mM) and for hydrolysis of Z-Gly-Leu-OH were 10.8 mM and 9.8 mM (cf ref. [3], bovine A, 33.0 mM; porcine A_1 and A_2, 35.0 mM), respectively.

These enzymes were relatively more stable than the bovine enzyme at high temperature, low pH and to action of urea. Effect of heat, pH and urea on enzymic activities of the human enzymes are shown in Table I.

Diisopropylphosphofluoridate (DFP) could not inhibit the enzyme activity of A_1 and A_2 even in excess amounts. The enzymic activity can be inhibited completely by 1,10-phenanthroline ($2 \times 10^{-3} M$, pH 8.0). A_1 and A_2 inactivated by dialysis against this reagent for 48 h regained activity only very slightly when dilute zinc chloride was added.

The substrate specificity of both fractions display the typical features of carboxypeptidase A. They hydrolyze the specific substrate A-B-C-OH at the position between B and C (A = benzyloxycarbonyl-, amino acid, peptide residue). Judging from preliminary experiments, the hydrolysis of Z-Gly-Phe-OH was a first-order reaction, therefore, C_1 values of other substrates were also calculated and listed in Table II. Among substrates tested, Z-Gly-Leu-OH was one of the best compounds for the enzymes, while bovine carboxypeptidase A has weakly hydrolyzed this substrate ($C_1 = 2.6$)[9]. As shown in Table II, both fractions were considerably free from other proteinases and peptidases such as trypsin (Bzl-Arg-NH_2), chymotrypsin (Ac-Tyr-OEt), leucine aminopeptidase (H-Leu-NH_2) and carboxypeptidase B (Bzl-Gly-Lys-OH)[10].

Table I. Effect of heat, pH, and urea on enzymic activities of carboxypeptidases A

Treatment		Original activity remaining		
		Human A_1 (%)	Porcine A_2[3] (%)	Bovine A[3] (%)
Heat	30 °C	100	100	100
(5 min	50 °C	81	92	45
pH 7.0)	57 °C	38	–	–
	60 °C	–	52	0
pH	4.0	64	65	0
(25 °C for	7.0	82	100	100
30 min)	8.0	100	100	100
	11.6	–	87	70
Urea	6 min	100	100	32
pH 8.0	11 min	100	100	12
at 25 °C)	20 min	100	97	5
	22 h	0	–	0

Assays of the human enzyme were performed with 0.02 M Z-Gly-Phe-OH in 0.02 M *Tris*-chloride-0.1 M NaCl, pH 7.8, at 25 °C. After 20 min the extent of hydrolysis was determined by measuring the release of amino groups with the ninhydrin reagent.

Table II. Action of human pancreatic carboxypeptidases A on synthetic substrates

Substrate[8]	C_1	
	A_1	A_2
Z-Gly-Phe-OH	1.18	2.68
Z-Leu-Phe-OH	0.087	0.22
Z-Gly-Leu-OH	1.63	4.33
Z-Gly-Pro-OH	0.002	0.006
Bzl-Arg-NH_2	0	0
Ac-Tyr-OEt	0	0
H-Leu-NH_2	0	0
Bzl-Gly-Lys-OH	0	0
Z-Leu-Phe-Val-Ala-OH ↓ ↓ ↓ 65 90 95% hydrolysed by A_1	S:E = 20:1, at 37 °C, pH 7.7 for 20 h	

Conditions were followed as described in Table I.

Zusammenfassung. Es sind einige physikochemische Eigenschaften der im menschlichen Pankreas in geringer Menge erhaltenen 2 Carboxypeptidasen A untersucht worden. Gewisse Unterschiede zu den Bovin- und Porcin-Carboxypeptidasen konnten festgestellt werden.

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[7] C_1, proteolytic coefficient in first-order reaction (min^{-1}). Measured by hydrolysis of benzyloxycarbonylglycyl-L-phenylalanine at pH 7.8 and 25 °C. Substrate concentration was 0.15 M, and enzyme concentration was 0.01–0.05 mg protein N/ml. Protein concentrations were estimated based on $E^{\%}_{280\ nm} = 19.0$.
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