Isoproterenol-stimulated cyclic AMP accumulation in fibroblast cultures from normal and diabetic subjects

Donor	Age	Years after diagnosis	Therapy	Culture passage No.	DPM [^{3}H]-cyclic AMP × 10^3 / mg protein	Group mean
MOD	46	6	Sulphonylurea	2	114 ± 13 (n = 5)	
MOD	49	5	Sulphonylurea	6	125 ± 18 (n = 22)	
MOD	54	6	Sulphonylurea	3,5	125 ± 44 (n = 15)	
MOD	57	3	Diet	2,4	141 ± 28 (n = 31)	126 ± 11
N	35			4,6	42 ± 15 (n = 59)	
N	36			2	53 ± 27 (n = 32)	
N	43			3	44 ± 6 (n = 20)	
N	50			6	79 ± 20 (n = 19)	55 ± 17

Difference between groups significant by Mann-Whitney rank sum test ($p = 0.05$). MOD, Maturity onset diabetics; N, normals.

per mg protein in cells from healthy subjects tended to increase in parallel with protein. Cells from maturity onset diabetics were consistently above control cells in cyclic AMP release.

In the range of 0.05–0.45 mg protein per dish a total of 203 observations were made on fibroblasts from 4 diabetics and 4 healthy subjects. Cells from the diabetic subjects displayed cyclic AMP levels significantly higher $(126 \pm 11) \times 10^3$, than those exhibited by the normal cells, $(55 \pm 17) \times 10^3$ (table).

Discussion. Two different methods of detaching cells from the culture dishes were used. Similar results for protein concentration were obtained. Dissolving the protein with 0.2 moles/l NaOH was less time-consuming than the mechanical method and was therefore preferred in most experiments. In the present study, differences in cyclic AMP accumulation could be the result of alterations in adenylate cyclase or phosphodiesterase activity. Phosphodiesterase inhibitor was not used, in order to avoid restriction of nucleotide efflux from cells[11]. Strict comparison of net cyclic AMP release by the [^{3}H]-adenine prelabeling method requires that uptake of tracer is the same in both cell groups and that cell content of ATP is likewise similar. Thus, the adenine prelabeling technique entails some limitations in the interpretation of the cyclic AMP data. However, it is evident that a difference in nucleotide metabolism exists between the two fibroblast groups.

In previous studies abnormalities in cell growth have been noticed in fibroblast cultures from diabetics. Cumulative number of fibroblast doublings have been decreased[2,3], and reduction in growth rate and cell density at confluence has also been noticed[12]. An association between increased cyclic AMP levels and growth cessation in cells has also been recognized[5]. The results in this study showing an increased cyclic AMP accumulation in fibroblast cultures from diabetics agree with what would be expected in cells which have a reduced capacity for cell proliferation. The results also indicate the presence of an intrinsic difference in nucleotide metabolism between 'normal' and 'diabetic' human cells. If present in other tissues as well, this deviation from the normal state may well be relevant for the understanding of diabetic metabolism.

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Effect of lectins from leguminous seeds on rat duodenal enterokinase activity

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Summary. Enterokinase activity from rat duodenal brush borders was assayed in vitro in the presence of purified lectins from 3 leguminous seeds. Noncompetitive inhibition of the enzyme was observed in each case. *Phaseolus* hemagglutinin was the most potent inhibitor among the 3 lectins tested.

Protein malnutrition observed in rats fed raw leguminous seeds is the result of imbalance in the amino acid pattern of the proteins and, above all, of the presence of numerous antinutritional and toxic components, which impair the protein digestibility[1,2]. Lectins may be responsible for this effect[3], just as trypsin inhibitors are[4]; the question still remains controversial[5]. Lectins could act by damaging enterocytes[6,7] or more specifically, by impairing the digestion of proteins and/or the absorption of amino acids[8]. Concerning protein digestion, every pancreatic protease activity we tested remained unaffected by lectins from leguminous seeds. But the enzyme required for the activation of pancreatic zymogens has not previously been tested. Enterokinase inhibitors have recently been found in numerous tubers and seeds[9], among which are several *Phaseolus* seeds. The only information reported about their nature is

that they could be separated from trypsin inhibitors by affinity chromatography. Since most enterokinases are membrane glycoproteins[10,11], the question arose as to whether lectins could account for the enterokinase inhibitor activity present in *Phaseolus* seeds[9].

Methods. Concanavalin A (Con A) was purchased from Miles Res. Laboratory and soybean lectin (SBA) (type VI) from Sigma. Whole PHA, i.e. the mixture of the 5 isolectins, was purified from *Phaseolus vulgaris* (var. Lingot blanc) seed by affinity chromatography; the ligand (heat-denatured porcine thyroglobulin) was coupled to CNBr-activated Sepharose 4B-Cl (Pharmacia) according to Itoh et al.[12]. Brush-borders were prepared as follows; duodenal mucosae from 6 rats were homogenized (Ultra Turrax TP. 18) for 30 sec at 4 °C. The homogenate was spun 4 times in 5 mM EDTA (pH 7.0) (600× g) for 10 min; $CaCl_2$ was then added until the EDTA was neutralized. Brush-borders were purified by successive centrifugations and finally resuspended in sodium maleate buffer. Enterokinase activity was assayed as described[13] using bovine trypsinogen (Fluka AG) as a substrate. Briefly, trypsinogen activation was initiated by adding 0.1 ml of trypsinogen solution (2 mg trypsinogen per ml of 5 mM HCl) to 1.4 ml of a medium containing brush-borders and the lectin in 15 mM sodium maleate buffer (pH 6.0). After 30 min, activated trypsinogen was determined by measuring the initial rate of hydrolysis of N-α-benzoyl-L-arginine-4-nitroanilide (Merck Co., FRG) at pH 8.2[14]. In all the experiments, PHA

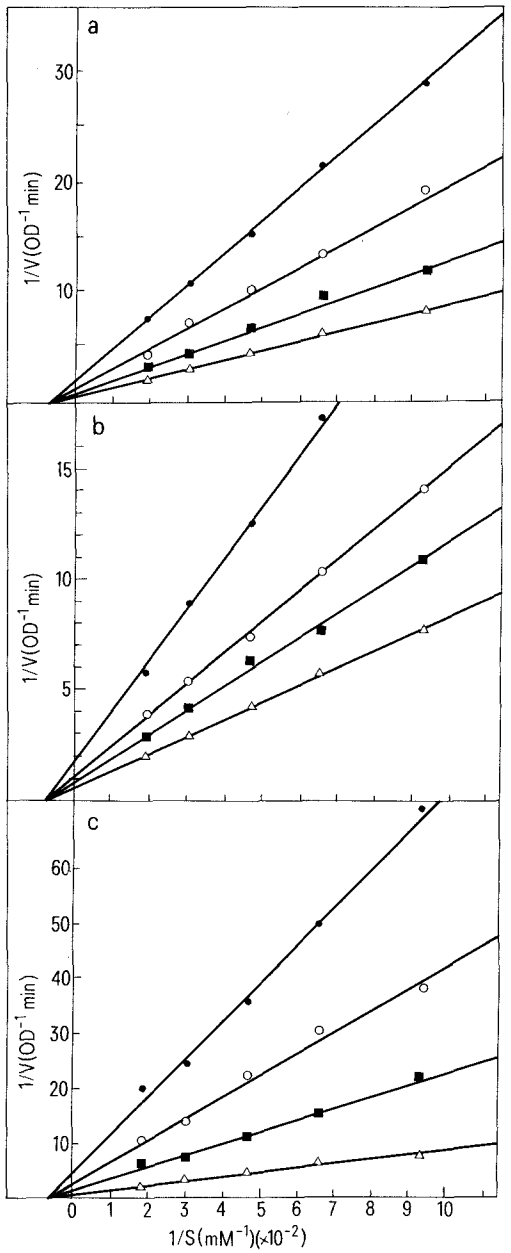

Figure 1. Double reciprocal plots of rat enterokinase activity. Velocities were measured according to Nordström and Dahlqvist[13] at 25 °C and pH 6.0 in maleate buffer; the overall ionic strength was 0.011 moles · l^{-1}. Various concentrations of trypsinogen were assayed in the presence of fixed concentrations of
a Con A (Δ, 0 μM; ■, 6 μM; ○, 15 μM ●, 30 μM).
b Soybean agglutinin (SBA) (Δ, 0 μM; ■, 2.7 μM; ○, 5.4 μM; ●, 13.5 μM).
c PHA (Δ, 0 μM; ■, 0.67 μM; ○, 1.34 μM; ●, 2.68 μM).

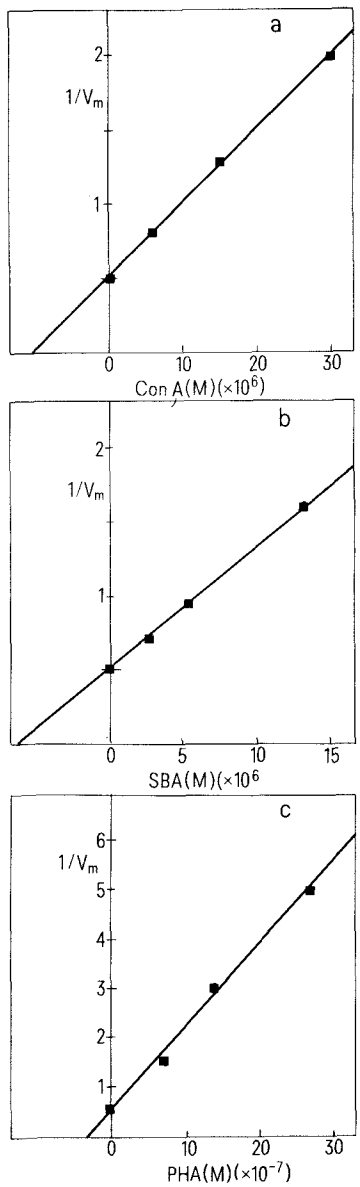

Figure 2. Replots of data from fig. 1: V_m^{-1} vs lectin concentrations. K_i values were determined from |lectin| -axis intercepts. *a* Con A; *b* SBA; *c* PHA.

concentration was measured spectrophotometrically according to Rasanen et al.[15] who gave $E^{1\%}_{280nm} = 11.4$ and $M_r = 126{,}000$ (tetramer). Kinetic parameters (K_m and K_i) were calculated by linear regression analysis.

Results. Results show that these lectins inhibit the enterokinase activity on duodenal brush border membranes. Lineweaver-Burk plots are summarized in figure 1; graphs derived from these data, i.e. V_m^{-1} vs lectins concentrations, are shown in figure 2. From these results, the 3 lectins appeared to act as fast-binding reversible inhibitors of rat enterokinase activity. Inhibitions were purely noncompetitive; Con A and soybean lectins appeared to be weak inhibitors (K_i respectively equal to 10^{-5} and $0.64 \cdot 10^{-5}$ M) whereas PHA appeared 20–30 times more potent ($K_i = 3.2 \cdot 10^{-7}$ M).

Discussion. Only human, bovine and pig enterokinases have been reported to be glycoproteins[10,11]. Little is known about the structure of their oligosaccharide moieties[16]; our results may thus provide indirect evidence that rat enterokinase is also a glycoprotein, a point that has not been verified yet. The K_i values found for Con A and soybean lectin fit with the K_d values for the binding of Con A with monomeric saccharides[17] and serum glycoproteins[18]. The K_i value found for PHA is 20 times greater than K_d values reported for its interaction with lymphocytes[18]. These K_i may thus reflect the apparent dissociation constant for lectin-enterokinase interaction. The non-competitive model of inhibition due to these 3 lectins from leguminous seeds is consistent with a mechanism of steric hindrance of the active site of rat enzyme.

In conclusion, we assume that the inhibition of rat enterokinase we observe in vitro may also occur in vivo; this could explain the etiology of the enterokinase deficiency syndrome previously described[19]. Thus, the lectins from leguminous seeds may contribute to the impairment of proteolysis in the digestive tract.

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Creatine phosphate inhibition of heart 5′-nucleotidase appears due to contaminants

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Summary. Creatine phosphate does not inhibit 5′-nucleotidase preparations from rat, dog or guinea-pig hearts. Previously reported inhibitory effects must have been due to contaminants present in some commercial preparations of creatine phosphate.

Inhibition of cardiac ecto-5′-nucleotidase (E.C. 3.1.3.5) by ATP or ADP has been observed over a decade ago, and speculations regarding the possible physiological importance of these effects with respect to the regulation of coronary dilatation and adenosine formation have been made[2-4].

More recently it has also been reported that creatine phosphate inhibits 5′-nucleotidase from rat heart and, in view of the key role played by this compound in myocardial energy transport, it was suggested that this effect may be of importance in the physiological control of 5′-nucleotidase activity and adenosine formation[5]. However, in the present study we have been unable to observe inhibition of cardiac 5′-nucleotidases by creatine phosphate, suggesting that observations to the contrary[5] were the results of contaminants known to be present in some commercial preparations of this phosphate ester[6-8].

Materials and methods. Hearts were excised from decapitated Sprague-Dawley rats (180–250 g) and guinea-pigs (300–400 g) of both sexes and then washed free of blood by retrograde perfusion via the aorta with ice-cold saline solution. Dog hearts were removed from mongrel dogs (13–16 kg) of both sexes, anesthetized with sodium pentobarbital (32 mg/kg) and washed as above. Ventricles were cut out, blotted, minced, and microsomal fractions were obtained by differential centrifugation according to the procedure of Dutta and Mustafa[9] with minor modifications. Thus polytron homogenization was performed in 0.25 M sucrose and the resultant microsomal 100,000 g pellet was suspended in 50 mM Tris-HCl buffer, pH 7.5. Final protein concentration was approximately 1–1.5 mg/ml. Fresh or stored (at −20 °C) microsomes were used in assays.

To solubilize 5′-nucleotidase, rat heart microsomes (1 ml) were suspended in 50 mM Tris-HCl buffer, pH 7.5, containing 1% deoxycholate (DOC), 1% Triton X-100 or 1% Zwittergent 3-14 (SB-14). After 4 h at 4 °C the suspension was dialyzed against the same buffer containing 0.05% of the respective detergent. A partially purified enzyme preparation from rat heart acetone powder was obtained ac-