Mucopolysaccharides of Brain and Viscera in Two Cases of Mucopolysaccharidosis

GUIDO A. F. VAN DESSEL, ALBERT R. LAGROU, JEAN-JACQUES R. MARTIN and WILFRIED S. H. DIERICK

Froehlich Brasseur Unit for Neurobiology and Neuropathology, RUCA-Born Bunge Association, UIA Department of Internal Medicine, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

A variety of mucopolysaccharidoses are known to exist, depending on the type of glycosaminoglycan accumulated (McKusick et al., 1965). Recent studies point to a deficiency of specific degradative enzymes (Constantopoulos & Dekaban, 1975). The mucopolysaccharides stored in tissues have only occasionally been analysed (Phillipart, 1972; Van Hoof, 1973). We have reported the lipid composition of brain and viscera in two cases (L.L. and D.C.) of mucopolysaccharidosis showing the clinical features of Sanfilippo disease (Van Dessel et al., 1976). The present communication deals with the mucopolysaccharide composition in those patients.

The tissues were first defatted by using chloroform/methanol mixtures at various ratios (by volume). The delipidated residues were further subjected to proteolytic digestion with papain or Pronase, whereafter the glycosaminoglycans were precipitated from the supernatant with cetylpyridinium chloride. Total mucopolysaccharide concentration was determined by the carbazole method as modified by Bitter & Muir (1962), with glucuronolactone as a standard. Iduronic acid was assayed by the naphthoresorcinol method (Teller, 1967). The analysis of the N-substituted hexosamines was performed in an amino acid analyser (Van Dessel et al., 1975). The electrophoretic patterns of the mucopolysaccharides on cellulose acetate were obtained by the procedure of Wessler (1968).

The colorimetric determination of uronic acid (Table 1) indicated an increase in glycosaminoglycan in all tissues (not in the brain of D.C.), being most pronounced in the liver. The N-substituted hexosamines were also present in much higher concentrations with regard to the control value (except for the brain of D.C.). In the patients' tissues only the presence of N-substituted glucosamine could be demonstrated, whereas in the control an average value of 3:2 was calculated for the molar ratio of N-substituted glucosamine/N-substituted galactosamine.

Electrophoresis of mucopolysaccharides on cellulose acetate in barium acetate buffer showed, for the viscera, an intense spot migrating ahead of the heparin standard, but clearly moving more slowly than the hyaluronic acid, chondroitin 4-sulphate, chondroitin 6-sulphate and dermatan sulphate standards. The electrophoretic behaviour of heparan sulphate (Wessler, 1968) is very similar to that of the unknown spot. The presence of the same component could also be demonstrated in brain, but hyaluronic acid still remained the main constituent. Densitometric measurements of the electrophoretograms showed a 3- (viscera) to 10-fold (brain) increase in the percentage distribution of heparan sulphate in the mucopolysaccharide pattern. From these results
Table 1. Glycosaminoglycan content in brain and viscera

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Patient L.L.</td>
<td>1.1</td>
<td>1.3</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Patient D.C.</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1) mg of uronic acid & g of defatted tissue; 2) is total N-substituted hexosamines (mg/g of defatted tissue); 3) is N-substituted galactosamine (% of total N-substituted hexosamines); 4) is N-substituted glucosamine (% of total N-substituted hexosamines). N.D., Not determined (because of lack of material).
one can conclude that the patients were suffering from Sanfilippo disease. The results are also in agreement with the glycosaminoglycan content and distribution in brain and viscera published by Constantopoulos et al. (1976).


Calcium Ion-Dependence of Sugar-Transport Regulation in Atrial Muscle

IVAN BIHLER and CHANDRAWEB SAWH

Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3

Several interventions causing an increase in the cytoplasmic Ca$^{2+}$ concentration have been shown also to stimulate the membrane transport of sugars in muscle and some other tissues. It has therefore been suggested (Bihler, 1972; Clausen, 1975) that Ca$^{2+}$ may play a role in the regulation of sugar transport and mediate the effect of various physiological and pharmacological modulators of this process.

We have therefore investigated how interference with normal Ca$^{2+}$ fluxes affects the regulation of sugar transport by several modulating factors. Isolated rat left atria were perifused and analysed as described before (Sawh & Bihler, 1976). The atria were perfused for 15 min with Krebs & Henseleit (1932) medium, pH 7.4, gassed with O$_2$/CO$_2$ (19:1) and modified to contain 1.25 mM-Ca$^{2+}$, 4.0 mM-sodium pyruvate, a mixture of $^{14}$C-labelled and unlabelled 3-methylglucose, and tracer amounts of $[^3]$H-inulin to serve as extracellular marker. In parallel experiments $^{45}$Ca was used instead of sugar. Radioactivity was determined by double-label liquid-scintillation counting. The results are expressed as percentage penetration ($\pm$S.E.M.), i.e. the concentration in the intracellular water is given as a percentage of the final concentration in the medium. All atria were fully quiescent, thus eliminating any effects of muscular contraction on sugar transport.

As shown in Table 1, perifusion in Ca$^{2+}$-free medium, with or without addition of the Ca$^{2+}$ chelator EGTA, strongly antagonized the stimulatory effect of insulin. This calcium-dependence of the insulin effect is consistent with earlier data from skeletal muscle (Bihler, 1972).

The experimental drug D-600 (methoxyverapamil; Knoll, A. G., Ludwigshafen, Germany) interferes with excitation-contraction coupling in cardiac muscle and is believed to act by inhibiting the inward-Ca$^{2+}$-flux component of the action potential (Fleckenstein, 1971). The data in Table 2 show that in resting atria this drug also significantly decreased $^{45}$Ca uptake in the presence and absence of insulin and under hyperosmotic conditions (100 mM-mannitol added to medium) or in the absence of potassium (K$^+$-free medium), two conditions where Ca$^{2+}$ uptake is increased. Although ineffective