CHROM. 5787

Thin-layer chromatographic demonstration of aspartylglycosylamine and a novel acidic carbohydrate in human tissues

The occurrence of aspartylglycosylamine (2-acetamido-1-(β'-L-aspartamido)-1,2-dideoxy-β-D-glucose (AADG)) in the urine of some mentally retarded patients has been reported[1]. This condition, aspartylglycosaminuria (AGU), seems to constitute a special clinical entity with characteristic clinical features and a decreased activity of aspartylglycosylamine amido hydrolase[2]. We reported earlier a chromatographic procedure for the isolation of AADG from urine[3], and in the present paper we demonstrate the presence of AADG and also of a novel acidic carbohydrate in the brain and liver of AGU patients.

Experimental

Small pieces of brain cortex and liver were obtained from one deceased AGU patient (female, age 29 years) and from two controls (male, 58 years, and female, 13 years). Free amino acids and peptides, including AADG, were extracted with 70 % ethanol[4]. To exclude possible artifacts, both brain and liver specimens were also homogenised in water (1:10 w/v) and deproteinized with 12.5 % trichloroacetic acid (TCA). In order to avoid even TCA-induced artifacts, parts of all samples were merely homogenized in water as above, dialysed overnight at 4°, freeze-dried and again

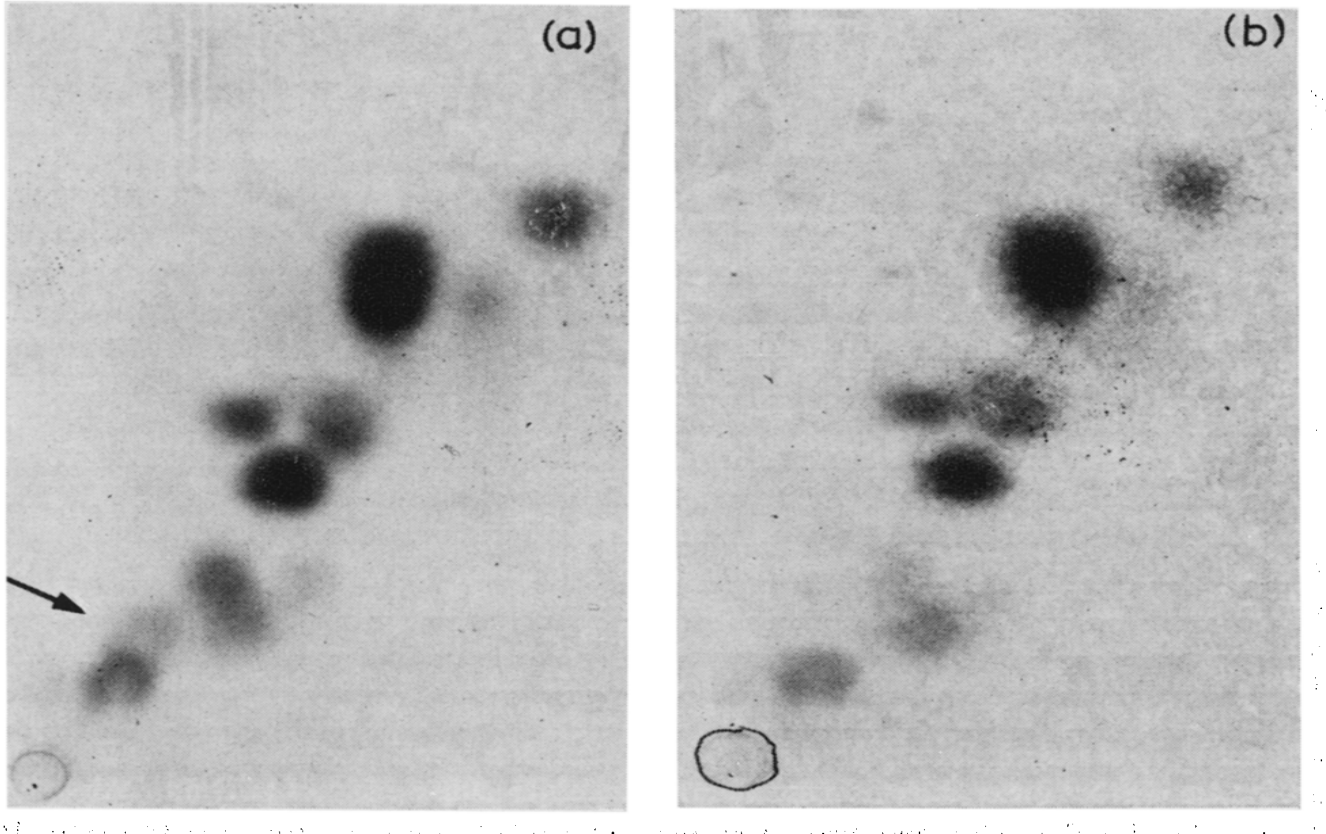

Fig. 1. Two-dimensional chromatograms of the free amino acids of brain cortical specimens. (a) Aspartylglycosaminuria, with a distinct AADG spot (arrow). (b) Control.

dissolved in water. All three types of sample were then subjected to the chromatographic procedure described below.

Prepared cellulose layers (E. Merck, Darmstadt, G.F.R.) were developed in *n*-butanol–acetone–acetic acid–water (35:35:10:20) (ref. 5) in the first dimension and in *n*-butanol–pyridine–ethyl acetate–water–acetic acid (50:10:20:20:20) (ref. 6) in the second dimension. The plates were stained with ninhydrin (0.5% in acetone). They were first heated at 45° for 5 min and then at 110° for a further 5 min.

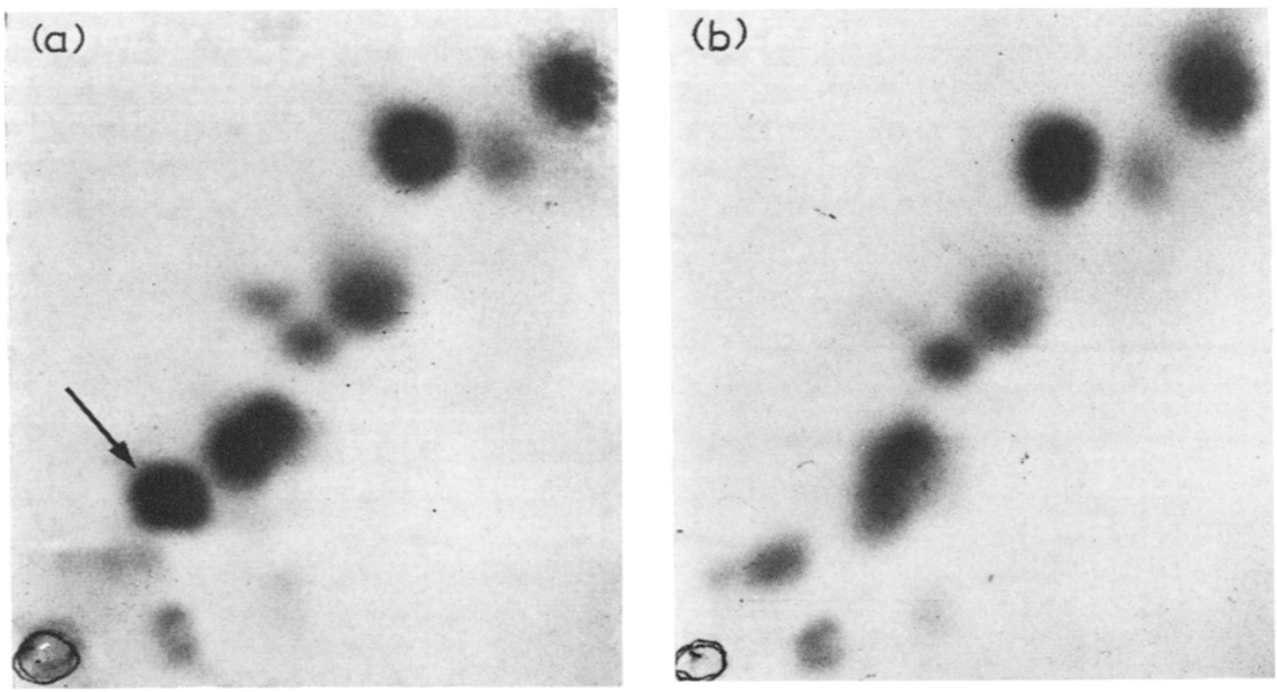

Fig. 2. Two-dimensional chromatograms of the free amino acids of liver specimens. (a) Aspartylglycosaminuria, with a strong AADG spot (arrow). (b) Control.

A distinct brown spot, corresponding to that of AADG, appeared after the first heating, and the colour assumed a more greenish-blue tint after the second heating. The R_F value of the spot was 0.08–0.05. It was detected in all AGU brain specimens but in none of the controls (Fig. 1). An identical spot was observed in all AGU liver specimens, and it was again missing from the controls (Fig. 2). Co-chromatography with pure AADG and the characteristic greenish-blue colour excluded all other peptides and amino acids. All three extraction methods gave similar results.

The same brain and liver samples were further chromatographed on prepared silica gel plates (E. Merck) using a one-dimensional run in *n*-butanol–acetic acid–water (50:25:25) (ref. 7) for 45 min, staining with a diphenylamine reagent[8] and heating for 20 min at 100°. This technique produced an unidentified brownish spot (R_F 0.13) that was missing from the control samples (Fig. 3). Isolation of this compound from the gel and its subsequent hydrolysis revealed that one of its constituents was again AADG, in addition to N-acetylneuraminic acid (NANA), hexosamine, and one or two bands in the area of uronic acid residues (in thin-layer chromatography). Its detailed biochemical structure is at present under more careful investigation. It has been found also in the urine of AGU patients[9].

Discussion

AADG was not detected in one AGU brain biopsy specimen when an automated amino acid analyzer (Unichrom, Beckman) was used[10]. This may be due to a lower sensitivity of the column chromatographic system for AADG, or to other differences in the analytical methods. Column chromatographic analysis has not yet been performed on the AGU autopsy specimens.

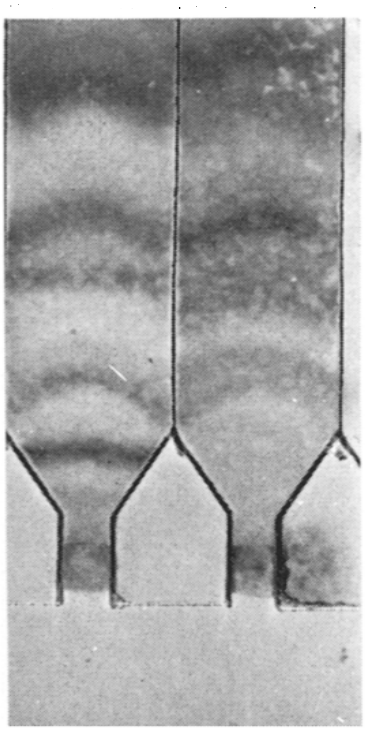

Fig. 3. One-dimensional chromatogram of the acidic carbohydrates of AGU (left) and control liver specimens. Note the extra band in the AGU liver (first band above the origin).

To our knowledge this is the first time that AADG has been shown to be present in human tissue, and it is probable that the acidic carbohydrate has never been characterized before. Together they seem to form the main lysosomal storage material in AGU, and their amounts in normal human tissues may be negligible or they may be completely absent.

The present findings again emphasize the value of simple but sensitive chromatographic methods in the detection and further characterization of biochemical abnormalities.

We thank Miss K. LINDBERG for technical assistance and the National Research Council for Medical Sciences and Kehitysvammaliitto r.y. for financial support.

Department of Neurology,
University of Helsinki,
Haartmaninkatu 4, Helsinki 29 (Finland)

J. PALO
H. SAVOLAINEN

1 F. A. JENNER AND R. J. POLLITT, *Biochem. J.*, 103 (1967) 48P.
2 R. J. POLLITT, F. A. JENNER AND H. MERSKEY, *Lancet*, 2 (1968) 253.

3 J. PALO AND K. MATTSSON, *J. Chromatogr.*, 50 (1970) 534.
4 G. PORCELLATI AND R. H. S. THOMPSON, *J. Neurochem.*, 1 (1957) 340.
5 R. S. ERSSER AND J. W. T. SEAKINS, *Nature*, 223 (1969) 1388.
6 M. BRENNER, A. NIEDERWIESER AND G. PATAKI, in E. STAHL (Editor), *Dünnschichtchromatographie, ein Laboratoriumshandbuch*, 2nd Ed., Springer-Verlag, Berlin, Heidelberg, New York, 1967.
7 B. A. LEWIS AND F. SMITH, in E. STAHL (Editor), *Dünnschichtchromatographie, ein Laboratoriumshandbuch*, 2nd Ed., Springer-Verlag, Berlin, Heidelberg, New York, 1967.
8 K. LAUNIALA, J. PERHEENTUPA, J. VISAKORPI AND N. HALLMAN, *Pediatrics*, 34 (1964) 615.
9 J. PALO AND H. SAVOLAINEN, *Clin. Chim. Acta*, 36 (1972) 431.
10 J. PALO, P. RIEKKINEN, A. U. ARSTILA AND S. AUTIO, *Neurology*, 21 (1971) 1198.

Received September 9th, 1971