MODIFICATION OF GLOMERULAR BASEMENT MEMBRANE FOLLOWING THE ADMINISTRATION OF THE LATHYROGEN AMINOACETONITRILE TO RATS

Sarah A. TAYLOR* and Robert G. PRICE+

Biochemistry Department, Queen Elizabeth College, University of London, Campden Hill, London W8 7AH, England

Received 23 April 1980

1. Introduction

Lathyrogens are a group of compounds which on ingestion induce a disease known as lathyrism [1]. The administration of β-aminopropionitrile, or its homologue, aminoacetonitrile, to laboratory animals results in osteolathyrism. Both these lathyrogens inhibit the activity of lysyl oxidase an enzyme important in the post-translational formation of inter- and intramolecular collagen crosslinks [2].

Glomerular basement membrane (GBM) is a member of the collagen family and contains collagen-like and polar regions [3]. When GBM is solubilised in sodium dodecyl sulphate (SDS) and 2-mercaptoethanol a residue, accounting for 20% of the total protein of the membrane, remains. Amino acid analysis demonstrated that the residue was collagenous and contained high amounts of glycine, hydroxyproline and hydroxylysine, while the material solubilised from the membrane contained a greater proportion of polar amino acids [4].

Glomerular nephropathies have been reported in patients with rheumatoid arthritis who were treated with D-penicillamine [5]. This drug, like aminoacetonitrile, interferes with collagen crosslinks [6] although it has a different mechanism of action [7]. It was of interest to determine whether aminoacetonitrile had an effect on the solubility and composition of GBM. Osteolathyrism was produced in rats by feeding aminoacetonitrile and its effect on rats of different ages compared. The amino acid composition, solubility and subunit pattern of GBM isolated from these rats is reported.

* Present address: Kennedy Institute, 6 Bute Gardens, Hammersmith, London W6 7DW, England

+ To whom reprint requests should be addressed

2. Materials and methods

Twenty-four male or female Sprague-Dawley rats (100 g body wt) were divided into 3 groups of 8 rats in each group. Aminoacetonitrile was given by gavage in doses of 31, 100 or 310 mg . kg^{-1} . day^{-1} to each of the groups for 10 consecutive days [8]. Rats receiving low or medium doses (31 or 100 mg . kg^{-1} . day^{-1}) gained weight normally, but rats receiving the highest dose (310 mg . kg^{-1} . day^{-1}) lost weight and fatalities were high. A slightly lower dose (250 mg . kg^{-1} . day^{-1}) was better tolerated and this dose was routinely given to groups of 10 rats.

GBM was prepared from pooled corteces separated from the kidneys of 10 rats [9] and the amino acid content determined [10]. Yields of GBM from lathyritic and normal rats were similar (20–25 mg/100 g cortex).

GBM (2.5 mg/ml) was solubilised by shaking at 37°C in 0.1 M sodium phosphate buffer (pH 7.1) containing 1% (w/v) SDS and 1% (v/v) 2-mercaptoethanol for 16 h [11]. The amount of membrane solubilised was determined following amino acid analysis of residue and soluble fractions prepared by low speed centrifugation. The pellet was washed with distilled water prior to acid hydrolysis and an aliquot (50 μl) of the soluble fraction hydrolysed [9].

The soluble components of GBM were separated on 5% polyacrylamide gels in 0.1 M sodium phosphate buffer (pH 7.1) containing 0.1% SDS [12]. Gels were stained in 0.25% Coomassie blue, destained in 10% acetic acid and scanned on a SP1809 spectrophotometer (Unicam, England). Markers from 5.3–26.5 × 10^4 mol. wt (BDH, England) were used to calibrate the gels.

3. Results

No significant differences were found in amino acid (table 1) or disaccharide content between GBM isolated from rats of different ages given a range of doses of the lathyrogen. All the values in table 1 fell within the normal range for rat GBM determined [13].

GBM from lathyritic rats proved to be more soluble in SDS and 2-mercaptoethanol than was normal membrane (table 2). Administration of 31 mg lathyrogen/kg body wt did not increase the solubility of GBM from young (100 g) rats, but when the dose was increased to 100 mg . kg^{-1} . day^{-1}, a small increase in solubility resulted. GBM prepared from rats receiving the highest does (310 mg . kg^{-1} . day^{-1}) was almost completely solubilised. The response to the lathyrogen was found to be age-dependent and a slightly lower dose (250 mg . kg^{-1} . day^{-1}) in 150 g rats produced only a small increase in solubility and no increase in 200 g rats.

No differences were found in the distribution of amino acids between the soluble and insoluble fractions prepared from GBM isolated from older rats (150 g and 200 g) and those of untreated rats. The insoluble fraction remained collagen-like while the soluble fraction contained a higher proportion of more polar amino acids. Amino acid analysis of the soluble fraction of GBM isolated from young severely lathyritic rats (100 g) indicated that a greater proportion of the amino acids considered to be representative of collagen had been solubilised. The increased solubility of the membrane in SDS and 2-mercaptoethanol was accompanied by an increase in the amount of 3- and 4-hydroxyproline, glycine and a smaller increase in hydroxylysine in the soluble fraction. There was also a decrease in the alanine and lysine present in this fraction. The amino acid composition of the soluble fraction isolated from 100 g lathyritic rats resembled that of whole GBM (table 1), a finding to be expected since >90% of the membrane was solubilised compared to only 75% in the older animals (table 2). The amounts of collagen-like material as indicated by glycine and hydroxyproline content fell in the residue of GBM of 100 g lathyritic rats compared to the residues remaining after solubilisation of membrane from 150 g and 200 g rats given lathyrogens.

The increased solubility of GBM isolated from 100 g lathyritic rats was characterised by a redistribution of the soluble components on SDS electrophoresis. The profile of components solubilised from normal rat GBM is given in fig.1b. Eight major and 7 minor bands of 3–30 × 10^4 mol. wt were present. The major component (band 6) had app mol. wt 15 × 10^4. The effect of lathyrogen on the molecular weight range of the components of soluble fraction

Table 1
Amino acid composition[a] of glomerular basement membrane prepared from rats of different ages given a range of doses of aminoacetonitrile (AAN)

Rat wt (g)	100[b]	100	100	200
Daily dose of AAN (mg/kg)	31	310	250	250
3HYP	9	9	8	11
4HYP	51	54	53	55
Asp	69	71	71	72
Thr	41	38	38	39
Ser	48	48	50	50
Glu	103	102	104	102
Pro	81	79	77	78
Gly	215	213	208	209
Ala	66	60	58	58
Cys	13	14	15	14
Val	41	46	47	45
Met	12	13	14	13
Ile	33	31	31	31
Leu	65	64	67	59
Tyr	16	17	17	17
Phe	29	28	30	30
His	20	21	21	21
Hyl	17	19	19	18
Lys	24	25	27	26
Arg	50	48	47	51

[a] Residues/1000 total amino acid residues
[b] The Sprague-Dawley rats used weighed 100 g, 150 g and 200 g and were 5, 6 and 8 weeks of age, respectively

Table 2
Solubility of glomerular basement membrane prepared from rats of different ages given a range of doses of aminoacetonitrile in SDS and 2-mercaptoethanol

Rat wt (g) at start of expt.	Dose of lathyrogen (mg/kg)	Soluble proportion[a] (%)
100	310	95
100	250	91
100	100	81
100	31	77
150	250	82
200	250	75
200	–	78

[a] Calculated from total nmoles amino acids recovered

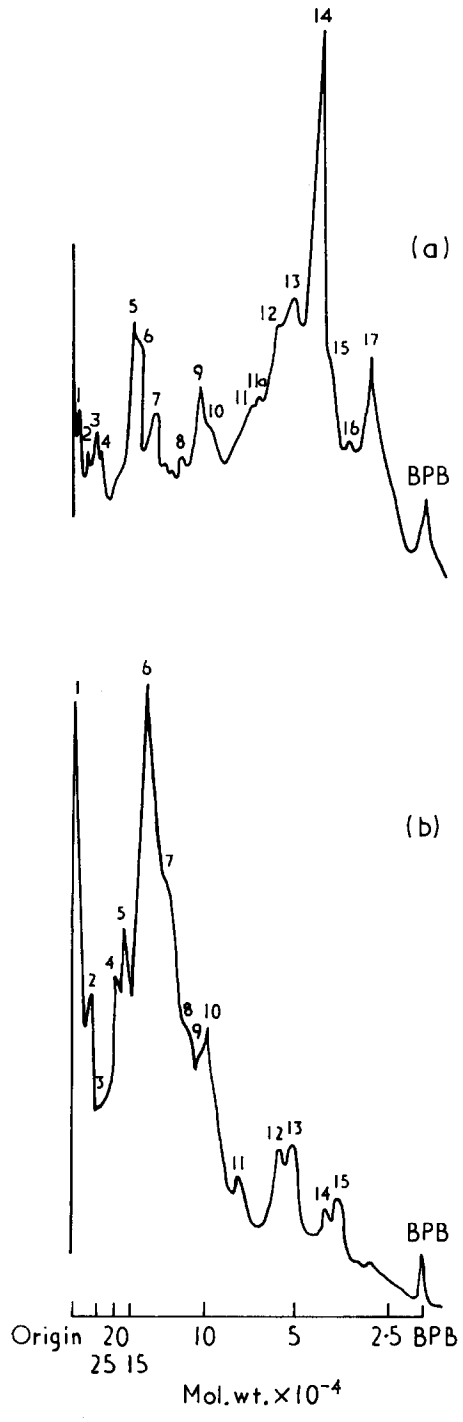

Fig.1. Comparison of the molecular weight distribution of glomerular basement membrane components from normal and lathyritic rats. Electrophoresis was carried out on 5% polyacrylamide–SDS gels as in the text. Densiometric scans were carried out at 560 nm with: (a) young rats given aminoacetonitrile (310 mg . kg^{-1} . day^{-1}); (b) normal untreated rats.

from GBM of lathyritic rats can be seen in fig.1a. The pattern was similar for both effective doses of the lathyrogen. Here the major subunit present had app. mol. wt 4.7×10^4 (band 14). There was a decrease in the amount of higher molecular weight material present and a concomitant increase in the amount of material migrating rapidly in the $3–7.5 \times 10^4$ mol. wt region. Two additional components with mol. wt $<3 \times 10^4$, namely 2×10^4 (band 17) and 2.7×10^4 (band 18) were present.

4. Discussion

No effect of feeding lathyrogens to rats on the solubility of the GBM was shown [14] but it should be noted that in [14] low amounts of lathyrogen were added to the diets of 125–150 g rats over 10 weeks. Our experiments demonstrate that the lathyrogen aminoacetonitrile is ineffective if the dose is too low and the animals weigh >150 g. The most significant changes were observed in young animals (100 g) actively synthesising GBM [9].

Collagen in the tissues of osteolathyritic animals can be more soluble than that in normal tissues. Examination of the solubilised collagen failed to demonstrate any intrinsic abnormalities except for the lack of intramolecular crosslinks [2]. We could show increased solubility of GBM but no apparent change in amino acid composition. Whether reducible crosslinks, similar to those found in collagen, are present in GBM is a matter of dispute since, although their presence has been reported [15], similar procedures have failed to detect them [3]. One explanation for this discrepancy may be that reducible crosslinks present in young animals are converted to non-reducible crosslinks in older animals [16]. Since lathyritic agents prevent crosslink formation and do not break them once formed, this would indicate that fewer crosslinks are formed in older rats or that a different type is present which is unaffected by aminoacetonitrile. This could explain our finding that aminoacetonitrile increased the solubility of GBM from young rats, particularly susceptible to the lathyrogen, and why the molecular weights of the solubilised subunits was lower in these animals. That these changes did not occur in older rats may be explained by the reduced rates of synthesis and the slow turnover of the glomerular basement membrane in older animals [9]. Even following treatment with lathyrogens some 5% of the

GBM remains insoluble and it is possible that this consists of helical chains and glycoproteins covalently bonded into an insoluble matrix by crosslinks unaffected by lathyrogens but which may be produced by an entirely different process [17,18].

These data provide indirect evidence for the presence in GBM of reducible crosslinks susceptible to lathyrogens in young rats.

Acknowledgements

The authors are grateful for financial assistance from the National Kidney Fund, England and the Wellcome Trust.

References

[1] Seyle, H. (1957) Rev. Can. Biol. 16, 1–82.
[2] Tanzer, M. L. (1965) Int. Rev. Connect. Tiss. Res. 3, 91–112.
[3] Spiro, R. G. (1976) Diabetologia 12, 1–14.
[4] Taylor, S. A. and Price, R. G. (1977) Biochem. Soc. Trans. 5, 236–237.
[5] Dische, F. E., Swinson, D. R., Hamilton, B. D. and Parsons, V. (1976) J. Rheumatol. 3, 145–154.
[6] Siegel, R. C. (1977) J. Biol. Chem. 252, 254–259.
[7] Nimmi, M. E. (1977) Proc. Roy. Soc. Med. 70, 65–72.
[8] Vogel, H. G. (1975) Connect. Tiss. Res. 3, 237–244.
[9] Price, R. G. and Spiro, R. G. (1977) J. Biol. Chem. 252, 8597–8602.
[10] Spackman, D. H., Stein, W. H. and Moore, S. (1958) Anal. Chem. 30, 1190–1206.
[11] Sato, T. and Spiro, R. G. (1976) J. Biol. Chem. 251, 4062–4070.
[12] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[13] Taylor, S. A. (1979) PhD Thesis, London.
[14] Kalant, N., Satomi, S., White, R. and Tel, E. (1977) Can. J. Biochem. 55, 197–206.
[15] Tanzer, M. L. and Kefalides, N. A. (1973) Biochem. Biophys. Res. Commun. 51, 775–780.
[16] Bailey, A. J. and Robins, S. P. (1973) Front. Matrix Biol. 1, 130–156.
[17] Rigby, B. J., Mitchell, T. W. and Robinson, M. S. (1977) Biochim. Biophys. Acta 79, 400–405.
[18] Robins, S. P. and Bailey, A. J. (1977) Biochim. Biophys. Acta 492, 408–414.