Factors affecting excretion of human urinary Tamm–Horsfall glycoprotein

K. L. LYNN*‡, A. SHENKIN† AND R. D. MARSHALL*

*Department of Biochemistry, University of Strathclyde, Glasgow, and †Department of Pathological Biochemistry, University of Glasgow, Glasgow, Scotland, U.K.

(Received 8 April 1981; accepted 23 July 1981)

Summary

1. Tamm–Horsfall glycoprotein was determined, by radioimmunoassay, in samples of urine from normal individuals under a variety of physiological conditions.

2. The amount of glycoprotein excreted in 24 h by our population (39 ± 13 mg, corrected for body surface area) was found not to be influenced by sex, age (19–60 years) or amounts of Ca²⁺, Mg²⁺ and Na⁺ excreted.

3. Urine samples collected at 2 h intervals over 24 h from individuals drinking in response to thirst, contained quantities of the glycoprotein which showed high positive correlations with urine volumes, but not with Ca²⁺, Mg²⁺ or Na⁺ excretion.

4. The amounts of urine and of the glycoprotein were correlated for individuals in anti-diuresis, induced by restriction of water intake. Relatively small amounts of glycoprotein were excreted by individuals in states of water-induced diuresis.

5. The amounts of glycoprotein excreted after exercise were positively correlated with the small volumes of urine voided, but they were uninfluenced by the degree of proteinuria or of hyaline cast formation.

6. The half-life for turnover of the glycoprotein in a given individual is highly variable, from a minimum of 3–7 to a maximum of 168 h.

‡ Present address: Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.

Key words: diuresis, glycoprotein, Tamm–Horsfall glycoprotein, urine.

Introduction

A glycoprotein, with the property of inhibiting haemagglutination in vitro brought about by certain myxoviruses, was shown by Tamm & Horsfall [1, 2] to be present in normal human urine. It is produced by the kidney [3], and ultrastructural studies have shown it to be associated with the plasma membrane of the luminal cells of the ascending limb of the loops of Henle and of the distal convoluted tubule as far as the junction with the initial collecting duct. It is not present on the maculae densae [4–7].

Its location corresponds precisely with the water impermeable section of the renal tubule, at least in the rat [8], and it is for this reason in part that a role for the glycoprotein in the maintenance of the relative water impermeability of the ascending limb was suggested [4, 5].

The Tamm–Horsfall glycoprotein is the most abundant protein of renal origin present in normal urine, and a number of procedures applied to small numbers of healthy individuals yielded results which suggested that up to 100 mg may be excreted daily [9–12]. In spite of these studies the factors involved in, and the mechanism of, the release of the glycoprotein from its association with the plasma membrane of the cell into the tubular urine are unknown. The nature of any involvement of Tamm–Horsfall glycoprotein in the diseased state [see 13] cannot be understood until these factors are resolved.
Methods

General

The isolation of Tamm–Horsfall glycoprotein, the raising of antiserum, and the radioimmunoassay for urinary Tamm–Horsfall glycoprotein were done as described previously [12, 14]. All samples were assayed in duplicate on three dilutions. Dilution was found not to affect the result obtained. The coefficient of variation in the assay was 13%.

Urine samples were collected into plastic containers and portions for analysis were stored at −20°C (those for Tamm–Horsfall glycoprotein in the presence of 0.02% sodium azide).

Standard Technicon Autoanalyser II methods were used for the analysis of creatinine (method no. SE4-0011 FH4), urea (method no. SE4-0001 FD4) and calcium (method no. SE4-0003 FJ4). Sodium and potassium were measured by flame emission photometry, and magnesium by atomic absorption spectrophotometry.

Urinary casts were counted in an improved Neubauer counting chamber (Hawksley Cristalite, Lancing, Surrey, U.K.) with a Wild M12 phase-contrast microscope with an MTr 23 transformer (Heerbrugg, Switzerland). Freshly voided urine (2 ml) was centrifuged (600 g, 10 min) in a conical-bottomed tube and all but 0.1 ml was removed. The pellet was resuspended in the remaining supernatant and the suspension was examined.

Urine osmolality was measured by freezing-point depression with an Advanced Osmometer (Advanced Instruments Inc., MA, U.S.A.).

Urinary collections

Tamm–Horsfall glycoprotein excretion was estimated on 24 h urine collections from 46 normal individuals (27 males, 19 females). Thirteen of these subjects took part in some or all of the following studies.

Five subjects made 2 h urine collections for Tamm–Horsfall glycoprotein measurements, in each case over the course of a 24 h period. During the time of the study no alcohol was consumed and no tobacco smoked. Strenuous exercise was avoided but there were no other restrictions on activity, nor on the consumption of food or fluid. The times of meals and periods of recumbency were noted. Three of these individuals repeated the study 6 months later under similar conditions.

Six subjects took part in a study to examine the excretion of Tamm–Horsfall glycoprotein both during anti-diuresis and during water-induced diuresis. During the study periods no tobacco was smoked and no alcohol consumed. On day 1, after a light breakfast, no fluid was consumed from 08.00 to 16.00 hours. Urine was collected at 10.00, 12.00, 14.00 and 16.00 hours. From 16.00 hours on day 1 to 08.00 hours on day 3 fluid intake was unrestricted. From 08.00 hours on day 3 at least 200 ml of fluid/h was consumed, to produce a diuresis. Urine collections were made as on day 1. Urine samples were divided into portions and used for the following estimations: Tamm–Horsfall glycoprotein, sodium, urea, and osmolality. A state of anti-diuresis was assumed where the osmolality was greater than 830 mosmol/kg, and of diuresis where it was less than 228 mosmol/kg.

The effect of exercise on Tamm–Horsfall glycoprotein excretion was studied in seven subjects. After a control 2 h urine collection (10.00 to 12.00 hours) and the collection of serum for creatinine estimation, the individuals undertook a variety of types of exercise for 20–40 min. The exercise consisted of running (two individuals), playing indoor hockey (three) or playing squash (two). Urine was then collected, provided that voiding was possible, at the end of exercise and at 30 min intervals until 90 or 120 min after the end of exercise. At no time was there any fluid restriction. Urine (2 ml) from each collection was immediately taken and used for the counting of urinary casts. The urinary pH and protein content were estimated with Labstix (Ames Co.) and aliquots were stored for the following estimations: Tamm–Horsfall glycoprotein, sodium, urea and creatinine.

Statistical treatment of data

Values for Tamm–Horsfall glycoprotein are expressed as means ± 1 SD (n = 6) and the difference between means were assessed by Student’s t-test. Correlation was calculated by least squares linear regression.

Results

Tamm–Horsfall glycoprotein excretion by normal individuals

The mean daily excretion of the glycoprotein by our normal population was 39 ± 13 mg/24 h corrected to 1.73 m² body surface area. Age over the range studied (19–60 years) was found not to influence the quantity excreted (r = 0.17), nor did sex difference. No correlation with 24 h urine volumes could be shown either for males and females or for the group as a whole (n = 49,
Diurnal variation in Tamm–Horsfall glycoprotein excretion

There was considerable variation in excretion of the glycoprotein, by individuals drinking in response to thirst, over the course of a 24 h period (Fig. 1). The mean excretion for the group for the 2 h periods was 3.6 ± 2.8 mg with a range of 0.36–18.2 mg. The pattern of excretion varied from one person to another, and in an individual on different occasions (Fig. 1). There was no circadian rhythm. Recumbency and eating did not influence excretion.

In general, as the 2 h urine volume increased so also did the amount of glycoprotein excreted (Fig. 1). For each of the individuals there was a positive correlation between the volumes of urine voided and the amounts of Tamm–Horsfall glycoprotein excreted (Table 1). The slope of the line differed from one individual to another over a range up to at least sixfold, and differed in one individual on the two separate occasions studied. Up to 79% of the variation in glycoprotein excretion may be accounted for by the volume of urine voided (Table 1).

The amounts of Ca$^{2+}$ and Tamm–Horsfall glycoprotein excreted were roughly proportional for one individual, subject no. 12, over one 24 h
TABLE 1. Regression coefficients for urine volumes (on x-axis as ml/min) and corrected amounts of Tamm–Horsfall (TH) glycoprotein excreted (on y-axis as mg/h) for 2 h periods over the course of 24 h

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>8</th>
<th>8</th>
<th>12</th>
<th>12</th>
<th>25</th>
<th>29</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>3.3</td>
<td>1.9</td>
<td>0.57</td>
<td>0.65</td>
<td>2.4</td>
<td>0.90</td>
<td>3.1</td>
</tr>
<tr>
<td>r</td>
<td>0.79</td>
<td>0.76</td>
<td>0.86</td>
<td>0.65</td>
<td>0.89</td>
<td>0.76</td>
<td>0.87</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>0.021</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine volumes (ml/24 h)</td>
<td>1470</td>
<td>980</td>
<td>2150</td>
<td>1840</td>
<td>1500</td>
<td>2390</td>
<td>1590</td>
</tr>
<tr>
<td>TH glycoprotein (mg/24 h)</td>
<td>54</td>
<td>44</td>
<td>49</td>
<td>44</td>
<td>55</td>
<td>50</td>
<td>64</td>
</tr>
</tbody>
</table>

FIG. 2. Log |Tamm–Horsfall (TH) glycoprotein excretion (mg/h)| as a function of log urine flow (ml/min) in the antidiuretic state (O) and in the water-induced diuretic state (●). The osmolalities of the urines in the former ranged from 833 to 1160 mosmol/kg, and in the latter were all less than 220 mosmol/kg. The regression equations were $a = 0.676 + 0.29$ ($n = 20$, $r = 0.76$) and $a = 0.186 + 0.24$ ($n = 9$, $r = 0.17$, P N.S.) for the antidiuretic and diuretic states respectively. For comparative purposes, the collective data for assays on 2 h urines over 24 h of individuals drinking in response to thirst are included (----, $a = 0.636 + 0.21$ ($n = 84$, $r = 0.68$)).

Tamm–Horsfall glycoprotein excretion under conditions of antidiuresis and water-induced diuresis

In antidiuresis, although the range of urine flows was small, there was a positive relationship calculated from the data in Fig. 2 between urine flow (UF, expressed in ml/min) and Tamm–Horsfall glycoprotein (TH, expressed in mg/h) excretion:

$$TH = 0.43 + 1.50UF \quad (r = 0.81, n = 20, P < 0.001) \quad (1)$$

In contrast to this, under conditions of water-induced diuresis there is a poor relationship of this nature ($r = 0.21$, P N.S.). At relatively high urine flows, but which were within the ranges of some of those seen in the diurnal study, the amounts of glycoprotein excreted were small (Fig. 2).

There was no correlation in either case between the amounts of glycoprotein excreted and the quantities of urea or Na+, either in absolute amounts or in concentrations, nor with the osmolalities or the total osmoles of the urines.

Tamm–Horsfall glycoprotein after exercise

In those individuals who voided urine immediately after the period of exercise the reduced urine flow at this time was invariably accompanied by a lowered excretion of Tamm–Horsfall glycoprotein. During the recovery period, there was in general a positive correlation between urine flow and excretion of Tamm–Horsfall glycoprotein (Fig. 3).

$$TH = -0.12 + 1.37UF \quad (r = 0.94, n = 22, P < 0.001) \quad (2)$$

when expressed in the same units as used in eqn. (1). The regression equation for those results where UF is $< 1$ is:

$$TH = 0.004 + 1.14UF \quad (r = 0.69, n = 17, P = 0.02) \quad (3)$$

The excretion of the glycoprotein was unrelated to either amounts or concentrations of urinary urea or Na+.

Urinary casts after exercise were predominantly hyaline, with occasional granular ones. No cellular casts or erythrocytes were found. The amounts or concentrations of Tamm–Horsfall glycoprotein in the urine and either the
number of casts excreted or the degree of proteinuria were totally unrelated (Fig. 3).

The assay for Tamm–Horsfall glycoprotein, made at the highest levels of proteinuria seen in these studies, was not affected by the amount of other urinary protein, as shown in control experiments with nephrotic urines (K. L. Lynn & R. D. Marshall, unpublished observation).

**Discussion**

The linear correlation between the volumes of urine voided and the amount of Tamm–Horsfall glycoprotein excreted over periods of time up to 2 h by normal subjects drinking in response to thirst, or in a state of antidiuresis or when recovering from exercise is of considerable interest. Analysis of the data of Bichler et al. [16] shows a similar trend for 1 h urines over 24 h, but these workers do not appear to have noticed this relationship. The limited data of Samuell [11], although we found similar urine flows are considered (Fig. 2). The values found for Tamm–Horsfall glycoprotein excretion during water-induced diuresis are not the maximum amounts possible.

Our measurements of Tamm–Horsfall glycoprotein included that comprising the casts [15, 22], and it was found that there was no correlation between the number of casts and the amounts of the glycoprotein excreted. This finding contradicts the earlier report of Patel [23], who used a less specific procedure for determining Tamm–Horsfall glycoprotein in the urine of individuals after exercise.

It has been reported that the amount of glycoprotein excreted in 24 h is directly related to the volume of urine passed [11], although we found in preliminary experiments [24] that, over 5 days, one individual excreted similar amounts of Tamm–Horsfall glycoprotein. The present studies show that the amount is often unrelated to urine volume and under some circumstances the amounts excreted may be the same even with differences in urine volumes of up to 2·5-fold. These results are understandable when we consider the values of the slopes obtained when glycoprotein excretion is plotted against urine volume (Table 1). For a given individual they may be the same or different on different days, but the reason is unknown.

The implications of the findings must await information concerning the rate of synthesis of the glycoprotein under a variety of conditions, its fate (particularly of that which is associated with cast counts between 1000 and 59 000 casts/ml. The degree of proteinuria was in all cases greater the higher the level of casts, as has been shown previously [15].

The rate of excretion of the glycoprotein may differ in a given individual over the course of a day by as much as 18-fold (Fig. 1). These findings suggest the need to re-examine the turnover time for the glycoprotein, which was reported to occur with a half life of 16 h, a value based on the assumption that the amount excreted over the course of the day was relatively invariant [17].

This marked variation in excretion of the glycoprotein (under non-stressed conditions) must be contrasted with the relative constancy of the rate of flow of urine in that region of the tubule where the glycoprotein is produced [4–7], where the walls of the tubule are largely impermeable to water [8]. Thus fluid enters the thick ascending limb of the loops of Henle in man at a minimum rate of 8–13 ml/min, based on the assumption that the [tubular fluid]/[plasma] ratio for inulin at the hairpin bend of the loop of Henle is of a similar order in man to those values found for the antidiuretic rat (7·4 [18]) and hamster (9–15 [19]) and the knowledge that the thin ascending limb is largely impermeable to water [19–21]. The maximum rate of entry to the thick ascending limb on the other hand cannot exceed 20 ml/min, which is the rate at which urine is voided in extreme diuresis. It seems unlikely therefore that the glycoprotein is washed passively from the walls of the renal tubule. The relationship of the type discussed above does not hold for individuals in a state of water-induced diuresis. In addition, the amounts excreted are lower than those found for individuals drinking in response to thirst, even when similar urine flows are considered (Fig. 2). The rate of flow of urine in that region of the tubule where the glycoprotein is produced [4–7, 14] and the knowledge that the thin ascending limb is largely impermeable to water [8] must be contrasted with the relative constancy of the rate of flow of urine in that region of the tubule where the glycoprotein is produced [4–7].
with the basal plasma membrane [4–71) and the amounts present, at the plasma membrane of the cells producing it, under different conditions. In any case, the reduced excretion of the glycoprotein in renal disease, that has sometimes been attributed to reductions in the number of functional nephrons [9, 11, 25], could arise in part from oliguria in some cases, but more work is required to examine this.

Acknowledgments

We thank the Wellcome Trust, the Medical Research Council and the National Kidney Research Fund for defraying the costs of the work. The co-operation of Mrs Mary Smith and Dr J. W. Dobbie in obtaining urine collections, and of Drs J. G. and W. A. Ratcliffe for the use of radioiodination facilities is gratefully acknowledged. K. L. L. was a Wellcome Research Fellow. We thank Dr M. R. Kibby for help with the statistical analyses, and Mr C. E. L. Shaw for assistance in preparing the Figures.

References