Renal dopaminergic system in nephrotic syndrome and after remission

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Abstract
Background. Although intrarenal dopamine is known to behave as an endogenous natriuretic hormone the role of the renal dopaminergic system in the sodium handling of nephrotic oedema remains unknown.

Study design. We monitored the daily urinary excretion of free dopamine, L-DOPA—its precursor, and its metabolites, DOPAC and HVA, during sodium retention accompanying the nephrotic state and natriuresis leading to oedema mobilization in eight patients (mean age 8.0 ± 2.4 years) with drug-induced remission of minimal-change nephrotic syndrome (MCNS).

Results. During natriuresis the urinary levels of dopamine did not increase in parallel with sodium excretion in any of the eight patients studied. Moreover, after remission of the nephrotic syndrome the urinary levels of dopamine were significantly lower than during the nephrotic state (1565.3 ± 361.7 vs 2416.1 ± 558.4, P = 0.02). In contrast, the urinary excretion of L-DOPA increased markedly during natriuresis resulting from remission of proteinuria (from 87.0 ± 40.5 up to 296.9 ± 86.3 nmol/24 h; P < 0.01).

Conclusion. We conclude that the natriuretic response resulting from drug-induced remission of proteinuria in MCNS is accompanied by a decrease in the renal uptake/decarboxylation of L-DOPA to dopamine.

Key words: minimal change nephrotic syndrome; natriuresis; dopamine; DOPAC; L-DOPA; noradrenaline

Introduction

The pathophysiology of oedema in the nephrotic syndrome is still unclear. The classic explanation for the development of sodium and water retention by the kidney in the nephrotic syndrome is based upon the loss of urinary protein and reduced plasma albumin, accompanied by reduced plasma oncotic pressure. Hypoproteinaemia causes a shift of intravascular fluid to the interstitial space, leading to peripheral oedema. As a consequence, effective circulatory plasma volume is reduced and vasoactive hormone systems responsible for central volume and blood pressure homeostasis are activated (the underfill theory) [1]. In recent years it has been proposed that intrarenal factors are primarily involved in sodium retention in the nephrotic syndrome (the overfill theory). It has been suggested that the defect is located in the distal nephron segments [2] and that the mechanism underlying increased reabsorption in the distal nephron is related to a blunted response to atrial natriuretic peptide [3].

Intrarenal dopamine has been recognized to behave as an endogenous natriuretic hormone [4]. Indeed, the renal production of dopamine increases in response to loading with sodium chloride [5] and the natriuresis that accompanies an acute or a chronic sodium load results, to a large extent, from dopamine produced by the renal proximal tubules [6,7]. However, the role of the renal dopaminergic system in the sodium handling of nephrotic oedema remains unknown.

The present study was aimed at measuring the daily urinary excretion of free dopamine, L-DOPA—its precursor, and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), before and after remission of the nephrotic oedema in patients with minimal-change nephrotic syndrome (MCNS). The urinary excretion of noradrenaline, which is known to be an important stimulus for sodium retention in the nephrotic syndrome [8,9], was also measured throughout the same time period. An account of some of these findings has been presented before [10].

Subjects and methods

Eight patients (2 females, 6 males; mean age 8.0 ± 2.4 years) were studied during recovery from MCNS. Remission was induced with prednisolone, 2 mg/kg/day (5 cases), 1.5 mg/kg/day (2 cases) and with the combination of prednisolone, 2 mg/kg/day plus cyclophosphamide, 3 mg/kg/day (1 case). During the study, for which the patients were hospitalized, no other medications were used except in two patients who received infusions of albumin (1 g/kg/day).
Table 1. The highest urinary levels of noradrenaline were characteristics of the study population throughout the renal sodium retention that characterises the nephrotic state. In contrast, urinary excretion of phosphorus did not differ between the nephrotic and natriuretic periods. General characteristics of the study population throughout the nephrotic and natriuretic phases are presented in Table 1.

During the nephrotic state because of symptomatic hypoalbuminaemia. On admission each patient presented with generalized oedema, a plasma albumin concentration below 20 g/l and a plasma creatinine not exceeding 110 μmol/l. Sodium intake was kept constant throughout the study, but varied individually from 60 to 90 mmol/day. Twenty-four-hour urine collections were obtained to assess the profile of the daily urinary excretion of L-DOPA, free dopamine, DOPAC, HVA and noradrenaline. The daily urinary excretion of 5-hydroxyindolacetic acid (5-HIAA), the main deaminated metabolite of 5-hydroxytryptamine was also measured. Urinary volume, body weight and daily urinary excretion of protein, sodium, potassium, phosphorus, creatinine and urine osmolality were also determined throughout the same time period. The 24-h urine specimens were collected with 15 ml of 6 M HCl, to prevent spontaneous oxidation of the amines and their metabolites. The levels of the catecholamines and their metabolites in urine were determined by means of high-pressure liquid chromatography with electrochemical detection (HPLC-ECD), as previously described [11]. Blood was collected every other day for measurement of plasma levels of total protein, albumin, sodium, potassium, chloride, BUN, creatinine, phosphorus, and osmolality. Urinary sodium and potassium were measured by means of a flame photometer (model A 6241, Radiometer, Copenhagen, Denmark) and urine and plasma osmolality were measured by means of an osmometer (Advanced Instruments, Inc., MA, USA, model 3 MO). Urinary and serum creatinine, phosphorus, and BUN were measured by a photometer (Hitachi Automatic Analyser, model 717).

**Statistical analysis**

To evaluate intragroup statistical significance we used the paired Student’s t test and ANOVA for repeated measurements. Regression analysis (Spearman) was used to evaluate the relationship between two variables. Results are given as mean ± SEM. Differences were considered statistically significant if the P value was ≤ 0.05.

**Results**

In each case, a progressive fall in proteinuria started within 5 days after the first dose of prednisolone or cyclophosphamide. This was found to precede the onset of natriuresis by 2–3 days. Three patients were capable of some sodium excretion while they were nephrotic. Yet in each case the sodium balance became clearly negative, the mean weight loss being 2.3 ± 0.4 kg (Table 1). Infusions of albumin in two patients were closely followed by 14- and 15-fold increases in urinary excretion of sodium, a twofold increase in urinary volume, and a simultaneous twofold increase in urinary protein excretion.

Urinary volume paralleled changes in urinary sodium excretion throughout the study and attained the highest levels during peak natriuresis (Table 1). In contrast, urinary excretion of phosphorus did not differ between the nephrotic and natriuretic periods. General characteristics of the study population throughout the nephrotic and natriuretic states are presented in Table 1.

As shown in Figure 1, urine dopamine output did not parallel the increase in urinary sodium excretion and was instead slightly reduced during peak natriuresis. Furthermore, the urinary excretion of dopamine after oedema mobilization was lower than during the nephrotic state. In contrast, the urinary excretion of L-DOPA increased markedly during natriuresis following the remission of proteinuria (Figure 1). This resulted in significantly reduced U/dopamine/L-DOPA ratios after remission of the nephrotic syndrome (Figure 1).

Similarly to what occurred with urinary dopamine, urine noradrenaline output attained the highest levels during the nephrotic state and decreased during natriuresis. The urinary excretion of noradrenaline was significantly lower at the end of natriuresis than during the nephrotic state (108.1 ± 49.8 vs 370.1 ± 163.8 nmol/24 h, P < 0.05).

The urinary levels of both DOPAC and HVA did not differ between the nephrotic and natriuretic phases (DOPAC, 5330.8 ± 927.4 vs 5870.6 ± 1578.1 nmol/24 h; HVA, 22 441.7 ± 8125.1 vs 18 040.2 ± 3189.8 nmol/24h). However, the U/DOPAC/U/DOPA ratios increased during natriuresis (3.8 ± 0.4 vs 2.4 ± 0.5, P < 0.05). No significant changes were observed in urinary 5-HIAA excretion between the nephrotic and natriuretic periods (21 034.4 ± 7390.6 vs 22 323.7 ± 5704.7 nmol/24 h).

**Discussion**

Given the important natriuretic role of renal dopamine, its involvement in the sodium handling of nephrotic oedema should be expected. In agreement with the underfill theory, it is anticipated that natriuretic hormones such as renal dopamine would be reduced during antinatriuresis and increased in parallel with the natriuresis resulting from the remission of proteinuria. On the other hand, the overfill theory implies that intrarenal defects during the nephrotic state are responsible for sodium retention. According to this theory, an impaired renal dopaminergic system activity could contribute to the increase in sodium reabsorption during the nephrotic state. The finding in this study of an enhanced renal dopaminergic system activity during nephrosis is against a direct role of endogenous dopamine in the natriuresis accompanying the remission of proteinuria in MCNS. It also fails to support a deficient activity of the renal dopaminergic system during the nephrotic state.

Similarly to what occurred with renal dopamine, the daily urinary levels of noradrenaline were lower at the end of natriuresis than during the nephrotic state. Those findings are in keeping with the observations of others that in some patients with MCNS the sympathetic nervous system is activated and the renal nerves are an important effector mechanism in the chronic renal sodium retention that characterises the nephrotic syndrome [8,9]. This is further reinforced by the finding that the highest urinary levels of noradrenaline were
Table 1. General characteristics of the study population during antinatriuresis (AN, lowest urinary sodium excretion during the nephrotic state), natriuresis (N, highest urinary sodium excretion during oedema mobilization and in basal conditions (B, 2 days after the stabilization of body weight following oedema mobilization).

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
<th>N</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²)</td>
<td>110.3 ± 10.9</td>
<td>137.8 ± 12.7*</td>
<td>124.6 ± 9.1</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg H₂O)</td>
<td>1029.9 ± 124.6</td>
<td>555.1 ± 70.0*</td>
<td>528.9 ± 39.0*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>32.7 ± 6.2</td>
<td>30.4 ± 5.9*</td>
<td>30.4 ± 5.9*</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24 h)</td>
<td>9.3 ± 3.3</td>
<td>163.1 ± 18.2*</td>
<td>69.9 ± 13.0*</td>
</tr>
<tr>
<td>Urinary potassium (mmol/24 h)</td>
<td>52.9 ± 3.4</td>
<td>56.9 ± 4.6</td>
<td>59.1 ± 6.9</td>
</tr>
<tr>
<td>Fractional excretion of sodium (%)</td>
<td>0.08 ± 0.04</td>
<td>1.53 ± 0.39*</td>
<td>0.44 ± 0.12*</td>
</tr>
<tr>
<td>Urinary volume (ml/24 h)</td>
<td>562.0 ± 148.6</td>
<td>1469.4 ± 156.7*</td>
<td>1143.4 ± 163.9*</td>
</tr>
<tr>
<td>Urinary protein (g/24 h)</td>
<td>3.1 ± 0.2</td>
<td>0.3 ± 0.2*</td>
<td>0.3 ± 0.2*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>109.5 ± 4.1</td>
<td>118.3 ± 5.6</td>
<td>114.0 ± 2.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.0 ± 3.5</td>
<td>63.8 ± 4.6</td>
<td>67.0 ± 4.2</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>16.9 ± 0.8</td>
<td>22.6 ± 1.1*</td>
<td>33.7 ± 1.2*</td>
</tr>
</tbody>
</table>

Significantly different in comparison with the corresponding values observed during AN (*P<0.01) and N (#P<0.01).

Fig. 1. Daily urinary excretion of (a) L-DOPA, (b) dopamine, and (c) U_dopamine/L-DOPA ratios in eight patients with minimal-change nephrotic syndrome during the nephrotic state (AN, lowest urinary sodium excretion), peak natriuresis (N, highest urinary sodium excretion), and at the end of natriuresis (B, two days after the stabilization of body weight following oedema mobilization. Significantly different in comparison with the corresponding values observed during AN (*P<0.05).

observed under conditions of enhanced sodium retention associated with severe hypoalbuminaemia.

Although it has been suggested that glucocorticoids have a complex effect on sympatheural and adrenomedullary outflows [12] no studies have evaluated the effects of these agents upon the renal dopaminergic system activity in humans. Evidence against the involvement of steroids on the changes in dopamine and L-DOPA excretion rates from the nephrotic to the natriuretic state is the following: (i) The dose of steroids was not changed throughout the nephrotic and the natriuretic periods in any of the eight patients studied; (ii) chronic steroid therapy has been associated with hyperdopaminuria, rather than a reduced excretion rate of dopamine [13]; (iii) Wolfovitz et al. [14] showed that rats treated with high doses of steroids did not show changes in L-DOPA excretion or in the renal production of dopamine at any given plasma level of L-DOPA, thus providing evidence that steroids do not directly influence the renal uptake of L-DOPA and its decarboxylation to dopamine in the renal cells of proximal tubules.

In contrast to what occurred with free dopamine, urine L-DOPA excretion increased markedly during the natriuretic response accompanying the remission of proteinuria. These findings are against the possibility that the renal synthesis of dopamine may have been impaired during natriuresis due to a reduced availability of the precursor to the kidney. It should be mentioned that the formation of dopamine in renal tubules is a two-step process involving the cellular uptake of L-DOPA at the apical and basolateral membranes followed by decarboxylation intracellularly [15]. Uptake of L-DOPA from the luminal side is an active process facilitated by the entry of sodium into the cells and seems to be the rate limiting step for intrarenal dopamine synthesis [16,17]. It has been suggested that salt loading increases the renal dopamine production by promoting the delivery of L-DOPA to sites of uptake by proximal tubular cells. Actually, in normal human subjects who changed from a low to a high sodium diet, an increase was observed in the urinary excretion of both L-DOPA and dopamine [6]. Therefore, the finding of a close relationship between the urinary excretion of sodium and L-DOPA with no accompanying changes in urine dopamine excretion provides evidence favouring the view that the increased excretion rate of sodium during remission of sodium.
proteinuria was accompanied by a decreased uptake of L-DOPA and/or decreased decarboxylation of L-DOPA to dopamine in proximal tubular cells. The increased production of renal dopamine during the nephrotic state should reduce proximal tubular sodium reabsorption in these conditions. This would be in agreement with clinical studies in nephrotic patients and experiments performed with animal models of nephrotic syndrome that suggested that the proximal reabsorption of sodium is decreased during the nephrotic state whereas an intrarenal defect promotes increased reabsorption of sodium in distal tubules [18,19]. The defect responsible for the increased distal sodium reabsorption during the nephrotic state has been attributed to impaired action of ANP. The resistance to ANP does not appear to be due to altered binding of the peptide to its receptors in the inner medulla but rather to a specific cellular change in the ANP signalling pathway, namely an increase in cGMP phosphodiesterase (PDE) activity that blunts the cellular actions of CGMP normally produced in response to ANP interaction with its biologically active receptor [1]. Accordingly, the specific cGMP PDE inhibitor zaprinast (M & B 22 948) normalized both the ANP-dependent cGMP accumulation and the natriuretic response in nephrotic kidneys [3]. Interestingly, previous studies from our group showed that incubation of renal slices with exogenous L-DOPA in the presence of M & B 22 948 resulted in a marked reduction in the accumulation of newly formed dopamine [20]. Since neither M & B 22 948 nor 8-bromo-cGMP altered the decarboxylation of exogenous L-DOPA in homogenates, it has been suggested that ANP-cGMP decreases the synthesis of dopamine in renal tissues, probably through a reduction of the uptake of L-DOPA into the tubular epithelial cells [20,21]. Thus, one may speculate from the changes in the urinary excretion of dopamine and L-DOPA in this study that the decrease in the \( U_{\text{dopamine}}/U_{\text{L-DOPA}} \) ratios when going from the nephrotic to the natriuretic phases may be related to the effects of ANP-cGMP on the synthesis of dopamine in renal tubules.

It is concluded that the natriuresis observed during drug induced remission of MCNS is accompanied by reduced uptake and/or decarboxylation of L-DOPA to dopamine in renal proximal tubules. These results are against a direct role of endogenous dopamine in the natriuresis accompanying the remission of proteinuria in MCNS and also fail to support a deficient activity of the renal dopaminergic system during the nephrotic status.

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References


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