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Blunted renal dopaminergic system activity in $HgCl_2$-induced membranous nephropathy

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Abstract

The present study evaluated the possible role of the renal dopaminergic system in the sodium retention of $HgCl_2$-induced nephrotic syndrome. The time courses of urinary excretion of sodium, protein, dopamine and the precursor L-3,4-dihydroxyphenylalanine (L-Dopa) were evaluated in $HgCl_2$-treated and control rats up to day 21. The renal aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the synthesis of renal dopamine, was evaluated during negligible proteinuria accompanied with enhanced sodium retention (day 7), increased proteinuria accompanied with greatest sodium retention (day 14) as well as during increased proteinuria accompanied with negative sodium balance (day 21). Also, the influence of volume expansion (VE, 5% bw) and the effects of the D_1-like agonist fenoldopam (10 μg kg bw^{-1} min^{-1}) on natriuresis and on proximal tubular Na^+,K^+-ATPase activity were examined on day 14. The daily urinary dopamine output and urinary dopamine/L-Dopa ratios were reduced in $HgCl_2$-treated rats from day 2 and beyond. This was accompanied by a marked decrease in renal AADC throughout the study. During VE, the fenoldopam-induced inhibition of proximal tubular Na^+,K^+-ATPase activity was similar between $HgCl_2$-treated and control rats. However, the urinary sodium excretion during fenoldopam infusion was markedly increased by 60% to 120% in control rats but was not altered in $HgCl_2$-treated rats. It is concluded that $HgCl_2$ nephrosis is associated with a blunted renal dopaminergic system activity. However, the lack of renal dopamine in $HgCl_2$ nephrosis does not appear to be related with the overall renal sodium retention in a state of proteinuria.

Keywords: Aromatic L-amino acid decarboxylase (AADC); Fenoldopam; $HgCl_2$; Na^+; K^+-ATPase; Nephrotic syndrome; Sodium handling; Renal dopamine; Volume expansion

Introduction

The features of the nephrotic syndrome in both man and experimental rat models are massive proteinuria and development of extra-cellular volume expansion due to enhanced renal sodium retention. Although the exact mechanisms involved in the enhanced sodium reabsorption in the nephrotic syndrome still remain to be fully elucidated, most of the available evidence implicates a primary distal renal sodium handling abnormality in this edema formation condition (Humphreys, 1994). It was suggested by Deschenes and Doucet (2000) that the mechanism responsible for the primary distal sodium retention in nephrotic syndrome is the combination of a blunted natriuretic response to atrial natriuretic peptide (ANP) (Plum et al., 1996; Rabelink et al., 1987) and an enhanced Na^+,K^+-ATPase activity in the cortical collecting duct (Deschenes and Doucet, 2000; Zolty et al., 1999). Recently, a primary sodium handling abnormality has been also invoked in the proximal tubules with the observation in nephrotic animals of a shift of the Na^+/H^+ exchanger NHE3 from the inactive to an active pool (Besse-Eschmann et al., 2002). However, the proximal tubular Na^+,K^+-ATPase activity was not examined (Besse-Eschmann et al., 2002) and, therefore, the role of the proximal tubules in the enhanced sodium retention in the nephrotic syndrome still remains to be elucidated.

Renal dopamine behaves as an endogenous natriuretic hormone by activating D_1-like receptors as a paracrine/autocrine substance (Cuche et al., 1976; Debska-Slizien et al., 1994; Jose et al., 1998). The epithelial cells of proximal tubules, but not of distal segments of the nephron, are endowed

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with a high aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the conversion of circulating or filtered L-3,4-dihydroxyphenylalanine (L-Dopa) to dopamine (Hayashi et al., 1990; Soares-da-Silva and Fernandes, 1990; Soares-da-Silva, 1994). This renal dopaminergic system appears to be highly dynamic and basic mechanisms for the regulation of this system are thought to depend mainly on the availability of L-Dopa, its fast decarboxylation into dopamine and in precise and accurate cell outward amine transfer mechanisms (Soares-da-Silva, 1994; Hussain and Lokhandwala, 1998; Aperia, 2000; Carey, 2001). During moderate sodium surfeit, dopamine of renal origin accounts for ~50% of sodium excretion (Siragy et al., 1989; Pelayo et al., 1983). Renal dopamine decreases tubular sodium reabsorption by inhibition of Na^+,K^+-ATPase activity directly or in response to the decrease in intracellular sodium following inhibition of Na^+–H^+ exchanger NHE3 (Siragy et al., 1989; Jose et al., 2000). Dopamine of renal origin can regulate sodium balance also by interaction with other natriuretic factors such as ANP (Aperia et al., 1996). In the late 1980s, several laboratories reported that the natriuretic response to ANP requires an intact renal dopaminergic system (Katoh et al., 1989; Rabelink et al., 1987). More recently, the interaction between ANP and renal dopamine was further reinforced by the findings that ANP and its second messenger, cGMP, cause a rapid translocation of the D_1-like receptors to the plasma membrane (Holtback et al., 1999). Interestingly, a decreased AADC activity in the proximal tubules was observed in puromycin aminonucleoside-induced nephrotic syndrome rat model (Sampaio-Maia et al., 2004).

On the basis of these considerations this study was undertaken with the aim of evaluating the possible role of renal dopaminergic system in the sodium retention observed in $HgCl_2$-induced membranous nephropathy. For this purpose we examined the time courses of the urinary excretion of sodium, protein, dopamine and the precursor L-Dopa in $HgCl_2$-treated and control rats. The rats were sacrificed during negligible proteinuria accompanied with enhanced sodium retention (day 7), increased proteinuria accompanied with greatest sodium retention (day 14) as well as during increased proteinuria accompanied with negative sodium balance (day 21) for the evaluation of the renal AADC activity. Also, the influence of volume expansion and the effects of the dopamine D_1 receptor agonist fenoldopam on natriuresis and on the Na^+,K^+-ATPase activity in the renal proximal tubules were examined during the phase of greatest sodium retention and ascites accumulation (day 14).

Materials and methods

In vivo studies

Mercury chloride-induced membranous nephropathy

Normotensive male Brown–Norway rats (Harlan, Barcelona, Spain), weighing 150–160 g, were selected after a 7-day period of stabilization and adaptation to blood pressure measurements. The animals received subcutaneous injections of 1 ml kg bw^{-1} of $HgCl_2$ (1 mg kg bw^{-1}) or the vehicle (NaCl 0.9%) on days 0, 2, 4, 7, 9 and 11.

Metabolic studies

The animals were kept under controlled environmental conditions (12 : 12 h light/dark cycle and room temperature 22 ± 2 °C); fluid intake and food consumption were monitored daily throughout the study. Two days before the first $HgCl_2$ or vehicle injection, the rats were placed in metabolic cages (Techniplast, Buguggiate-VA, Italy). The $HgCl_2$-treated and control rats had free access to tap water. The $HgCl_2$-treated rats were fed ad libitum throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 g kg^{-1} of sodium. In order to achieve the same daily sodium intake between the two groups, the control rats had only access to the mean daily rat chow intake of the $HgCl_2$-treated animals. Twenty-four hours urine were collected on uneven days in empty vials, for later determinations of protein, creatinine and sodium, and on even days in vials containing 1 ml hydrochloric acid 6 M (to avoid the spontaneous oxidation of the amines) for later determination of catecholamines. Urine volume was gravimetrically determined. Blood pressure (systolic and diastolic) and heart rate were measured daily throughout the study in conscious restrained animals, between 7.00 and 10.00 AM, using a photoelectric tail-cuff pulse detector (LE 5000, Letica, Barcelona, Spain). Four determinations were made each time and the means were used for further calculation.

Animals were sacrificed during negligible proteinuria accompanied with enhanced sodium retention (day 7: $HgCl_2$, $n=6$; vehicle, $n=6$), increased proteinuria accompanied with greatest sodium retention (day 14: $HgCl_2$, $n=12$; vehicle, $n=10$) as well as during increased proteinuria accompanied with negative sodium balance (day 21: $HgCl_2$, $n=9$; vehicle, $n=7$). On the days of sacrifice after the 24 h urine collection for determination of sodium and creatinine, the animals were anaesthetized with pentobarbital sodium (50 mg kg bw^{-1}; ip) and the ascites volumes were measured through moistening and weighing an absorbent paper. Blood was collected from the heart in tubes containing heparin and lithium/heparin for later determination of plasma catecholamines and biochemical parameters, respectively. Thereafter, the kidneys were rapidly removed, weighed and the outer cortex isolated. Fragments of renal cortex were used later for determination of AADC and Na^+,K^+-ATPase activity in proximal tubular cells. Other fragments of renal cortex weighing around 200 mg were placed in vials containing 1 ml of 0.2 M perchloric acid, stored at −80 °C until quantification of catecholamines by HPLC with electrochemical detection. Segments of jejunum ~10 cm in length were also removed, opened longitudinally with fine scissors and rinsed free from blood and intestinal contents with cold saline; thereafter, the jejunal mucosa was removed with a scalpel for later determination of AADC activity.

Volume expansion

In another set of experiments, fourteen days after first $HgCl_2$ or vehicle injection, the animals were anaesthetized with pentobarbital sodium (50 mg kg bw^{-1} followed by 20 mg kg