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Original article

Expression of the non-erythroid Rh glycoproteins in mammalian tissues

Expression des glycoprotéines Rh non érythroïdes dans les tissus de mammifère

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Abstract

A novel family of proteins, the Mep/AMT/Rh glycoprotein family may mediate important roles in transmembrane ammonia transport in a wide variety of single-celled and multicellular organisms. Results from our laboratory have examined the expression of the non-erythroid proteins, Rh B Glycoprotein (Rhbg) and Rh C glycoprotein (Rhcg), in a wide variety of mammalian tissues. In the kidney, Rhbg and Rhcg are present in distal nephron sites responsible for ammonia secretion. In the mouse kidney, Rhbg immunoreactivity is exclusively basolateral and Rhcg immunoreactivity is exclusively apical, whereas in the rat kidney Rhcg exhibits both apical and basolateral expression. Chronic metabolic acidosis increases Rhcg expression in the outer and inner medulla of the rat kidney; these changes, at least in the outer medullary collecting duct, involve changes in total cellular protein expression in both principal and intercalated cell and changes in its subcellular localization. In the liver, Rhbg is present in the basolateral plasma membrane of the perivenous hepatocyte and Rhcg is present in bile duct epithelia. In the gastrointestinal tract, Rhbg and Rhcg exhibit cell-specific, axially heterogeneous, and polarized expression. These patterns of expression are consistent with Rhbg and Rhcg mediating important roles in mammalian ammonia biology. The lack of the effect of chronic metabolic acidosis on Rhbg expression raises the possibility that Rhbg may function either as ammonia sensing-protein or that it may mediate roles other than ammonia transport.

Keywords: Rh glycoprotein; Mouse; Rat; Kidney; Liver; Stomach; Instestinal tract

Mots clés : Glycoproteine Rh ; Souris ; Rat ; Foie ; Estomac ; Tractus intestinal

1. Introduction

A wide variety of evidence suggests that the Mep/AMT/Rh glycoprotein family mediates important roles in ammonia [1] metabolism and transport. Three mammalian members of this family are known, Rh A glycoprotein (RhAG/Rhag), Rh B Glycoprotein (RhBG/Rhbg) and Rh C Glycoprotein (RhCG/Rhcg). RhAG is an erythroid-specific protein present in the Rh complex of erythrocytes [1,2]. Both heterologous expression studies and studies using erythrocytes from RhAG-null individuals show that RhAG can transport ammonia [3–7]. Rhbg and Rhcg are non-erythroid Rh glycoproteins [8,9]. Functional studies has confirmed that both Rhbg and Rhcg transport ammonia, although different studies have identified differing affinities for the two molecular forms of ammonia, NH_3 and NH_4^+, and whether transport is electroneutral or electrogenic [10–16]. In this review, we will concentrate on the expression of Rhbg and Rhcg in the kidney, liver and gastrointestinal tract.

2. Expression in the mouse kidney

The kidney is a major site of ammonia metabolism and transport. Ammonia is produced by the proximal tubule and

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[1] The term ammonia is used to refer to the combination of the two molecular species, NH_3 and NH_4^+. When referring specifically to the molecular species NH_3, we specifically state "NH_3", and when referring to NH_4^+ we specifically state "NH_4^+."

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is preferentially secreted into the luminal fluid [17,18]. Apical secretion is probably mediated by NHE-3 [19], although a mechanism independent of NHE-3 may be operable [20]. Ammonia is reabsorbed by the medullary thick ascending limb of the loop of Henle predominantly via transport by the apical Na^+-K^+-$2Cl^-$ cotransporter, NKCC-2 [18]. Ammonia is then secreted by the collecting duct into the luminal fluid through mechanisms that appear, in general, to involve net NH_3 transport [18]. Approximately 80% of total urinary ammonia is secreted between the micropuncturable distal tubule and the tip of the collecting duct [17,21].

The initial cloning of Rhbg and Rhcg reported that their mRNA was expressed in the kidney and that they exhibited substantial predicted secondary structural homology with the Mep/AMT family of proteins [8,9]. Thus, it was postulated that Rhbg and Rhcg might be important in renal ammonia metabolism [8,9]. Shortly thereafter, we generated antibodies directed against the cytoplasmic carboxyl-terminus of Rhbg and Rhcg that we used to examine mouse renal Rhbg and Rhcg expression [22]. Immunoblot analysis confirmed that the mouse kidney expressed both proteins. Rhbg exhibited basolateral labeling in the connecting segment (CNT) and in the majority of initial collecting tubule (ICT) and cortical collecting duct (CCD) cells. In the outer medullary collecting duct (OMCD) and inner medullary collecting duct (IMCD) only a subpopulation of cells exhibited basolateral immunoreactivity. Essentially similar findings were reported by others in the rat kidney [23]. Colocalization of Rhbg with cell-type specific markers demonstrated Rhbg immunoreactivity in CNT cells, A-type intercalated cells, non-A, non-B cells and in CCD and ICT principal cells. In the ICT and CCD, A-type intercalated cells, but not B-type intercalated cells, expressed basolateral Rhbg immunoreactivity. In the OMCD and IMCD, only intercalated cells exhibited Rhbg immunoreactivity. Rhcg was expressed in the same epithelial cells, with the exception that mouse OMCD principal cells expressed Rhcg, but did not express detectable Rhbg. RhCG immunoreactivity was apical, thereby complementing the basolateral Rhbg expression. These findings suggested that Rhbg and Rhcg mediate cell-specific roles in ammonia transport and/or signaling.

3. Functional characterization of collecting duct ammonia transport

The observation of ammonia transporter family members in apical and basolateral membranes of collecting duct cells suggested that they would contribute to transepithelial ammonia secretion. Several studies have shown that collecting ducts secrete ammonia, and that the rate of secretion parallels the transepithelial NH_3 gradient, and is independent of the transepithelial NH_4^+ gradient [18,24]. Although the Na^+-K^+-$2Cl^-$ cotransporter, NKCC1, is present in the collecting duct, it does not contribute significantly to ammonia secretion [25]. Thus, ammonia secretion involves net NH_3 transport in parallel with active H^+ secretion. Net NH_3 transport can indicate either passive NH_3 diffusion across lipid bilayers, facilitated NH_3 transport or NH_4^+/H^+ exchange.

We examined whether collecting duct ammonia transport involved passive lipid-phase diffusion or specific transport activities by reasoning that the rate of diffusive transport would be proportional to the ammonia concentration, whereas transporter-mediated transport would exhibit saturable kinetics [26]. To examine the mechanisms of collecting duct ammonia transport we used the mIMCD-3 collecting duct cell line grown on permeable support membranes, enabling separate apical and basolateral plasma membrane domains, and we quantified uptake of the ammonia analog, [^{14}C]methylammonia ([^{14}C]MA). Basolateral MA transport exhibited both diffusive and transporter-mediated components. Transporter-mediated uptake predominated at concentrations below ~7.0 mM, exhibited a K_m for MA of ~4.6 mM and was competitively inhibited by ammonia with a K_i of ~2.1 mM. This basolateral transport activity was not mediated by Na^+-K^+-ATPase, Na^+-K^+-$2Cl^-$ cotransporter, K^+ channels or KCC proteins, and did not involve Na^+ or K^+. Altering membrane potential did not alter transport, consistent with electroneutral transport. Changing the H^+ gradient altered transport in a pattern consistent with either NH_4^+/H^+ exchange or facilitated NH_3 transport. Finally, mIMCD-3 cells expressed basolateral Rhbg immunoreactivity. Thus, the mIMCD-3 collecting duct cell exhibits a basolateral transport activity consistent with facilitated NH_3 transport or NH_4^+/H^+ exchange and likely to be mediated by Rhbg.

We then examined apical ammonia transport [27]. Apical MA transport exhibited both diffusive and saturable, transporter-mediated kinetics. The apical transport activity had a K_m of ~7.0 mM and ammonia was a competitive inhibitor with a K_i of ~4.3 mM. Transport activity was bidirectional, linked to H^+ gradients, unaltered by membrane voltage, did not involve Na^+ or K^+, and was not mediated by H^+-K^+-ATPase, Na^+-K^+-ATPase or Na^+/H^+ exchange. Finally, mIMCD-3 cells expressed apical Rhcg. These results identify that the renal collecting duct mIMCD-3 cell has an apical transport activity consistent with facilitated NH_3 transport or NH_4^+/H^+ exchange, and possibly mediated by Rhcg.

Thus, the major components of both apical and basolateral ammonia transport across the mIMCD-3 collecting duct cell is via specific, transporter-mediated mechanisms likely to involve Rhbg and Rhcg. The lower affinity of the apical transporter is consistent with luminal ammonia concentrations being higher than interstitial ammonia concentrations. Moreover, the affinity of Rhcg for ammonia is less than that of Rhbg [10]. The identification that the apical and basolateral transport activities were compatible with net NH_3 transport, either facilitated NH_3 transport or NH_4^+/H^+ exchange, is consistent with findings that bacterial ammonia transporter family member, AmtB, is an NH_3 transporter [28–30]. However, there was trans-stimulation of the apical transport activity, suggesting an NH_4^+/H^+ exchange transport activity which can also function in an NH_4^+/NH_4^+ exchange mode [27]. More important, however, is that collecting duct apical and basolateral ammonia transport occurs via a saturable and inhibitable, transporter-mediated mechanism.