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NH_4^+ modulates renal tubule amantadine transport independently of intracellular pH changes

Kerry B. Goralski [a,f], Ratna Bose [a], Daniel S. Sitar [a,b,c,d,e,*]

[a] Department of Pharmacology and Therapeutics, University of Manitoba Winnipeg, Manitoba, Canada R3E 0W3
[b] Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
[c] Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada
[d] Centre on Aging, University of Manitoba, Winnipeg, Manitoba, Canada
[e] Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada
[f] College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada

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Abstract

A bicarbonate-dependent organic cation transporter, unique from rOCT1 and rOCT2, primarily mediates amantadine uptake into renal proximal tubules. We examined whether intracellular pH regulates bicarbonate-dependent amantadine transporter function in these tubules. NH_4Cl treatment resulted in immediate intracellular alkalinization of tubules for up to 30 s followed by gradual acidification that was maximal at 5 min. Proximal tubule amantadine uptake was similarly inhibited (60%) by NH_4Cl during both the early intracellular alkalinization and later acidification phases. Sodium propionate treatment resulted in immediate intracellular acidification of proximal tubules without inhibiting amantadine uptake. NH_4Cl inhibition of bicarbonate-dependent amantadine uptake was dose-dependent, competitive and sex-dependent. NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ inhibited amantadine uptake into proximal tubules similarly. NH_4Cl also stimulated efflux of amantadine and tetraethylammonium from preloaded proximal tubules, suggesting mediation of a facilitated process. These data suggest the potential for direct modulation of organic cation transporters by NH_4^+ in rat kidney proximal tubules.

Keywords: Amantadine; Ammonium ion; Bicarbonate; Kidney; Organic cation transport

1. Introduction

Net secretion of organic cations by the kidney involves transport across the basolateral and luminal membranes of proximal tubules. The first step, passage of organic cations into the proximal tubule cells is facilitated by the basolateral membrane organic cation transporters OCT1 and OCT2 (Grundemann et al., 1994; Karbach et al., 2000; Lee and Kim, 2004; Sweet et al., 2000). In addition to OCT1 and OCT2 rat and rabbit proximal tubules contain at least one additional transporter that is selective for the organic cation amantadine (Goralski et al., 2002; Goralski and Sitar, 1999; Wright and Dantzler, 2004; Wright et al., 2004). Although the amantadine transporter has not yet been molecularly identified, it has been well characterized by its substrate selectivity and regulatory control mechanisms that differ significantly from rOCT1 and rOCT2 (Escobar and Sitar, 1995; Escobar et al., 1994; Goralski et al., 2002; Goralski and Sitar, 1999). The similarity of amantadine transport in rat and human renal cortical slices strongly suggests that the amantadine transport process is intact and important in human kidney (Wong et al., 1990; 1992b).

The second step in organic cation secretion involves transport of the cationic drug across the luminal membrane via the organic cation/H^+ exchanger (Rafizadeh et al., 1987; Takano et al., 1984; Wright and Wunz, 1988). Multidrug and toxin extrusion transporter 1 (MATE1) has been cloned recently from human and mouse, is highly expressed in the kidney, is localized in renal tubule luminal membranes, and functions as an organic cation/H^+ exchanger (Otsuka et al., 2005). The

* Corresponding author. Department of Pharmacology and Therapeutics, University of Manitoba, A220-770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. Tel.: +1 204 789 3532; fax: +1 204 789 3932.
E-mail address: sitar@cc.umanitoba.ca (D.S. Sitar).

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findings of Otsuka and coworkers strongly suggest that the MATE1 transporter is the putative organic cation/H^+ exchanger that mediates the final step of organic cation secretion in the kidney. We have limited information regarding amantadine transport across the luminal membrane of proximal tubules (Wong et al., 1992a). Candidate transporters for luminal amantadine transport include MATE1 and the recently identified OCTN1 and OCTN2 carnitine/organic cation exchangers (Wu et al., 1999; Yabuuchi et al., 1999).

The present study represents a continuation of our focus on acid/base perturbation and renal tubule amantadine transport (Escobar et al., 1995; Escobar et al., 1994; Goralski and Sitar, 1999; Goralski et al., 1999). At the outset, the main goal of this study was to determine the effects of intracellular pH on the renal proximal tubule transport of amantadine. Amantadine accumulation into isolated proximal tubules was evaluated in the presence and absence of NH_4Cl and sodium propionate, which alkalinize and acidify pH_i, respectively (Bose, 1995; Kim and Dantzler, 1995, 1997; Roos and Boron, 1981). Our study has identified that the urinary acidifier NH_4Cl impairs amantadine renal proximal tubule accumulation while enhancing its efflux. Importantly, the modulatory effect of NH_4^+ on amantadine renal tubule transport was independent of pH_i and was consistent with direct inhibition of uptake via the bicarbonate-dependent amantadine transporter and enhanced amantadine egress, possibly via an amantadine/NH_4^+ exchange mechanism.

2. Materials and methods

2.1. Renal tubule preparation

The experimental procedures involving the use of animals have been approved by the University of Manitoba Protocol Management and Review Committee. Separation of proximal tubules was performed by the Percoll density gradient centrifugation method (Gesek et al., 1987; Vinay et al., 1981) as modified and previously reported in detail by our laboratory (Escobar et al., 1994; Goralski and Sitar, 1999; Wong et al., 1991). For each experiment, renal proximal tubules isolated from 4 rats were pooled for transport assays. Data characterizing the degree of separation of proximal tubules with our methodology have been previously reported in detail (Escobar and Sitar, 1995; Wong et al., 1991). Enzyme assays demonstrate that alkaline phosphatase activity is dominant in proximal tubules and is consistent with their reported distribution in the nephron (Gesek et al., 1987; Scholer and Edelman, 1979; Vinay et al., 1981).

2.2. Measurement of pH_i in isolated proximal tubule segments by fluorescence microscopy

We used the pH-sensitive fluorescent dye 2′7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) to measure the effects of 20 mM NH_4Cl and 30 mM sodium propionate on pH_i in isolated proximal tubules. Similar procedures have been used by others to study changes in pH_i in isolated renal tubules (Kim and Dantzler, 1997; Martinez et al., 1997). Fluorescence microscopy experiments were performed with a Nikon Diaphot TMD inverted microscope equipped with an epifluorescence attachment and a Nikon fluor oil immersion objective (50×) (Nikon Inc. Melville, NY, USA). The excitation light was generated by a mercury vapor light source. Wavelength-specific filters for measuring pH were set at 440 (isosbestic wavelength for BCECF) and 490 nm (the excitation maximum). The emitted fluorescence light passed through the dichroic mirror and a 540 nm emission filter, and was captured and digitized by a Digital Imaging Camera (The Cooke Corporation, Auburn Hills, MI, USA). Axon Ion Imaging Workbench software (Axon Instruments Inc., Foster City, CA, USA) was used for acquisition and processing of images, and for ratiometric and non-ratiometric imaging. All software, camera and instruments were supplied by Optikon Corporation Ltd. (Kitchener, Ontario, Canada).

The pH studies were carried out in oxygenated (95% O_2; 5% CO_2) Krebs–Henseleit Solution (KHS) (pH 7.4) containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 1.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11 mM glucose. The pH_i experiments were carried out using a single concentration of NH_4Cl (20 mM) and sodium propionate (30 mM), and in proximal tubule fragments from male rats, as those concentrations have been established to alter intracellular pH. To prepare tissue for imaging, 50 μl of proximal tubule suspension (8–10 mg/ml) were diluted into 930 μl of oxygenated (95% O_2; 5% CO_2) KHS. The dilute tubule suspension was then dispensed overtop of an ultra thin fluorescent grade cover slip inside a temperature-controlled bathing chamber (Biophysica TCV-2 digital temperature regulator and chamber, Baltimore, MD, USA) on the stage of the microscope. The dilute tubule suspension was allowed to incubate for 15 min during which time the tubules normally adhered to the glass cover slip without any further treatment or manipulation of the slide surface. 20 μl of 1 mM BCECF-AM (the ester form of the dye; final concentration 20 μM) were added to the bathing chamber and the tubules were allowed to incubate for 45 min at 25 °C to load the dye. BCECF-AM enters the cells readily, where it is then hydrolyzed by nonspecific esterases to the impermeable fluorescent dye BCECF. Upon completion of the dye-loading period, the extraneous dye was washed away with oxygenated (95% O_2; 5% CO_2) KHS buffer by a gravity-fed flow through system set at a flow rate of 5 ml/min for 5 min. The buffer flow was then stopped and the system was allowed to equilibrate for 15 min. A 5 min control baseline was established prior to the administration of 20 mM NH_4Cl or 30 mM sodium propionate in oxygenated (95% O_2; 5% CO_2) KHS buffer. The NH_4Cl or sodium propionate treatments were administered via the flow through system (5 ml/min for 2 min), after which the flow was stopped and the tissue was allowed to incubate for a further 10 min. NH_4Cl and sodium propionate were then washed away with fresh oxygenated (95% O_2; 5% CO_2) KHS buffer via the flow through system (5 ml/min for 5 min) followed by an additional 15 min equilibration period, control baseline, second treatment period (NH_4Cl or sodium propionate) and washout. Thus, each tissue preparation was exposed to both 20 mM