Bull Environ Contam Toxicol (2012) 89:699–703
DOI 10.1007/s00128-012-0756-y

Effect of Sulfhydryl Modification on Rat Kidney Basolateral Plasma Membrane Transport Function

Rais A. Ansari · Syed A. A. Rizvi · Kazim Husain · Anastasios Lymperopoulos · William O. Berndt

Received: 22 May 2012 / Accepted: 17 July 2012 / Published online: 2 August 2012

Abstract Transport processes are the hallmark of functioning kidney. Various nephrotoxicants disrupt the transport processes to manifest nephrotoxicity. Of several nephrotoxicants, mercuric chloride ($HgCl_2$) depletes the reduced glutathione (GSH) in kidney and has been observed to affect the in vitro *p*-aminohippurate (PAH) transport by basolateral (BL) membrane vesicles. The role of renal nonprotein sulfhydryls such as, reduced GSH has been demonstrated to affect the PAH transport by BL membrane vesicles. The role of protein sulfhydryls in transport process of PAH by BL membrane is not known. Due to mercury mediated effects on sulfhydryls, the effects of protein-sulfhydryls (–SH) modifying reagents in the current study were investigated on PAH transport by BL membrane. It was observed that modification of –SH by *p*-chloromercuribenzoate sulphate (*p*CMBS), and mercuric chloride ($HgCl_2$) decreased while recovering the protein –SH with dithiothreitol treatment provided protection against the effects of *p*CMBS, and $HgCl_2$ on PAH transport by BL membrane vesicles.

Keywords Transport · Membrane function · Sulfhydryls · Mercuric chloride

Transport of solutes and organic substances is the hallmark of functioning kidney (Esteva-Font et al. 2012). Many nephrotoxicants have been studied over the decades to understand the mechanism of their action (Baggett and Berndt 1986; Klonne and Johnson 1983; McDowell et al. 1976; Zalme et al. 1976). Many of these studies have not addressed the cellular and subcellular insults, which results in acute tubular necrosis leading to acute renal failure. One of the major concerns is that how a number of toxicants affecting the tubular epithelial function lead to same sequence of events leading to nephrotoxicity. The failure of functioning kidney after nephrotoxicants, suggests a common mechanism beside diverse nature of nephrotoxicants. Like the specific role of mitochondria in toxicity and metabolic syndrome, plasma membrane vesicles in the form of functioning transport processes can also provide the opportunity as the subcellular target of nephrotoxicants (Houston 2011; Lenaz 2012; Scatena 2012). The availability of enriched preparation of basolateral (BL) plasma membrane vesicles from renal cortical tissues has made it possible to evaluate the role of sulfhydryls on specific transport process (Boumendil-Podevin and Podevin 1983; Jensen and Berndt 1988). A wide variety of toxicants especially nephrotoxicants cause toxicity by oxidative stress which can alter both the non-protein and protein –SH status of cells that can contribute to altered function (Lash 2011). Effects on transport processes due to nephrotoxicants in acute renal failure have been observed (Hori et al. 1985; Lee et al. 1990). In the present study, the protein –SH level was reduced by treatment with *p*CMBS, and $HgCl_2$ and their effects on PAH transport by BL membrane

William O. Berndt: Published posthumously to honor who passed away in 2005.

R. A. Ansari (✉) · S. A. A. Rizvi · A. Lymperopoulos
Department of Pharmaceutical Sciences, College of Pharmacy Health Professions Division, Nova Southeastern University, 3200 South University Drive, Fort Lauderdale, FL 33328-2018, USA
e-mail: ra557@nova.edu

K. Husain
Department of Physiology, Pharmacology and Toxicology, Ponce School of Medicine, PO Box 7004, Ponce, PR 00732-7004, USA

W. O. Berndt
Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68102, USA

vesicles isolated from kidney cortex were studied. Further effects of reducing agent, dithiothreitol (DTT) in counteracting the effects of *p*CMBS, and $HgCl_2$ on PAH transport by BL membrane vesicles were also studied. It was observed that effects of reduction of membrane associated –SH caused reduction in transport of PAH by BL membrane vesicles while treatment with DTT was able to reverse the effects offering protection against the –SH modifying agents including $HgCl_2$.

Materials and Methods

The right side out BL membrane vesicles were prepared by modification of the method of Boumendil-Podevin and Podevin (Boumendil-Podevin and Podevin 1983). The protein content of the plasma membrane was determined by dye binding method of Bradford (Bradford,1976). The membrane preparation was diluted to 2.5 mg protein/mL with buffered sucrose solution. Marker enzymes were used to monitor the relative purity of the BL membranes. Na^+/K^+ ATPase activity was enriched fivefolds–ninefolds in BL membranes relative to kidney cortex homogenate.

The BL membrane vesicles and sheets (after lysing membrane vesicles into water as mentioned in transport measurement) were treated with *p*CMBS, and $HgCl_2$ for 10 min and the –SH level was measured by Ellman's reagent, 5,5′-Dithio-bis (2-nitrobenzoic acid) (DTNB). A standard of reduced glutathione (GSH) was used to determine the sulfhydryl values expressed as reduced GSH (Baker et al. 1990).

Transport of [^{14}C] *p*-aminohippurate ([^{14}C] PAH) into BL membrane vesicles was assayed at 37°C into a total volume of 60 µL. BL membrane vesicles were preloaded with PAH (200 µM) after 30 min preincubation with PAH and repelleting the membranes in ultracentrifuge as described earlier followed by reuspension in sucrose-Tris buffer solution (Ansari et al. 1990). BL membrane vesicles of 50 µg protein were added to each vial in 20 µL aliquots, followed by buffered sucrose (270 mM sucrose-20 mM Tris–HEPES). Transport was initiated by addition of labeled [^{14}C] PAH. Transport was abrogated after 15, 30, 60, and 600 s by filtering 50 µL aliquots through 0.45 µm filters. The transport was stopped by washing the filters with 6.0 mL ice-cold solution containing 130 mM NaCl, 20 mM Tris containing 5 mM probenecid.

The filters were dried and dissolved in 5.0 mL scintillation fluid. The corrections for the amount of the radioactivity bound to membrane sheets and filters were done by incubating 20 µL of membrane protein in 0.36 mL of water for 5 min, followed by additional 5-minute incubation after addition of 20 µL labeled substrate. After filtration as mentioned above, the radioactive binding to filter and membrane was determined by counting the filters. Since the net transport values varied from preparation to preparation, the results are expressed as the percentage of the equilibrium, which was achieved and determined at 600 s. To ascertain that equilibrium was achieved at 600 s, the preliminary experiment measurements were performed at 20 and 30 min. The values of 20 and 30 min did not differ from 600 s measurements.

The effects of *p*CMBS, and $HgCl_2$ on PAH transport by BL membrane vesicles were determined by exposing the BL membranes with *p*CMBS, and $HgCl_2$ for10 min. For determining the effects of DTT on transport process of PAH by BL membrane vesicles, initially, membranes were treated with *p*CMBS, and $HgCl_2$ for 10 min. These agents were removed after centrifugation of the membrane. DTT was added to membrane vesicle for 30 min. After removal of DTT by centrifugation, the transport studies were performed for set period of times as mentioned in graphs.

The data are expressed as mean ± standard error of five or six membrane preparations. The data were subjected to one-way analysis of variance (ANOVA). The individual differences among means were determined by student's *t*-test.

Results and Discussion

The effects of *p*CMBS and $HgCl_2$ treatments on sulfhydryl levels of BL membrane vesicles and sheets are presented in Table 1. The –SH modifying agents, *p*CMBS and $HgCl_2$ were able to decrease the –SH status of BL membrane vesicles and sheets. Both *p*CMBS and $HgCl_2$ treatment resulted in statistically significant reduction of –SH levels of BL membranes and sheets. The nephrotoxicant, $HgCl_2$ was able to deplete significantly the –SH level of basolateral membrane vesicles and sheets like other –SH modifying reagent, *p*CMBS.

Table 1 SH status of BL membrane vesicles and sheets of control and treatment groups

Group	Membrane vesicles	Sheets
Control	38.03 ± 6.49	40.38 ± 6.67
10 µM *p*CMBS	25.19 ± 3.00*	22.25 ± 9.12*
100 µM *p*CMBS	15.63 ± 4.26**	6.99 ± 7.97**
Control	34.74 ± 4.69	37.14 ± 4.88
10 µM $HgCl_2$	23.03 ± 5.96*	13.62 ± 5.51*
100 µM $HgCl_2$	15.07 ± 7.12**	8.48 ± 2.16**

Data are presented as mean (nmoles GSH/mg protein) ± SD of 5 observations. * $p < 0.001$ and ** $p < 0.0001$

The effects of *p*CMBS, and $HgCl_2$ on PAH transport by BL membrane vesicles are presented in Figs. 1, 2 and 3. Initial treatment of membrane vesicles with *p*CMBS at 5 and 10 μM did not significantly reduce the overshoot value of transport (60 and 120 s transport values) (Fig. 1). However, later treatment of BL membrane vesicles with *p*CMBS and $HgCl_2$ at 10 μM, significantly reduced the percent transport values at 60 and 120 s. The protective effects of DTT treatment on *p*CMBS, and $HgCl_2$ mediated effects on PAH transport by BL membrane are summarized in Figs. 2 and 3 respectively. The DTT treatment of vesicles for 30 min after 10 min pretreatment of vesicles with *p*CMBS, and $HgCl_2$ offered protection to PAH transport by BL membrane. DTT was able to reverse the effects of *p*CMBS, and $HgCl_2$. It is noteworthy that DTT not only restored the overshoot at 2 min of PAH transport but also increased the overshoot level from control.

Isolation of membrane vesicles has provided the opportunity to study the effects of nephrotoxicants on membrane functions especially on the transport process. It also, provides the opportunity to study the effects of toxicants on transport processes devoid of metabolic effects. Often the toxicants affect the metabolic process that disrupts the energy process involved in active transport processes. Earlier, our studies have demonstrated that nephrotoxicants disrupt the transport processes of both BL and brush border (BB) membrane vesicles (Ansari et al. 1990). One of the nephrotoxicants, $HgCl_2$ is capable of binding with proteins –SH and C=O groups and affects the transport of PAH by BL membrane vesicles when studying in vitro exposure and transport studies (Ansari et al. 1990). However, when animals were exposed with $HgCl_2$, it affected Na^+/glucose transport by BB membrane vesicle after 16 h of exposure (Ansari et al. 1990). In order to evaluate the mechanism of effects of $HgCl_2$, we utilized –SH modifying agents to evaluate the effects on PAH transport process by BL membrane vesicle. It was observed that both *p*CMBS and $HgCl_2$ significantly reduced the amount of –SH which is expressed as reduced GSH level (Table 1). Treatment with *p*CMBS, and $HgCl_2$ also

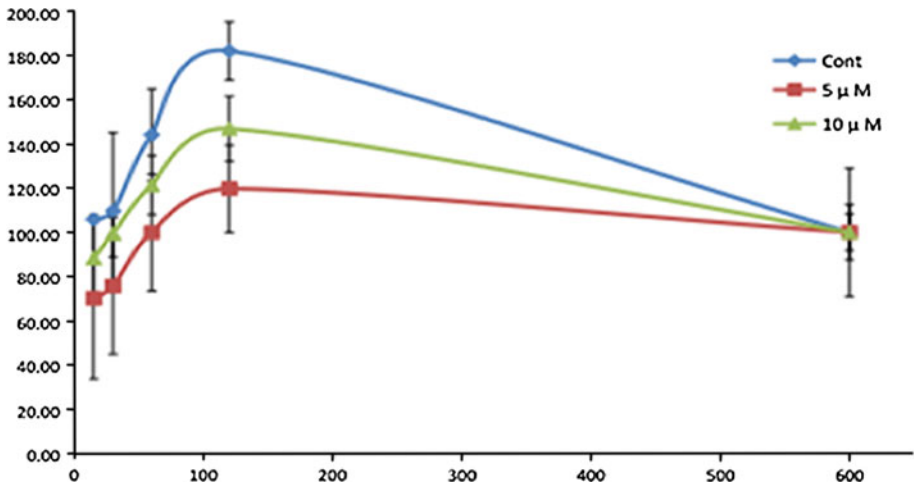

Fig. 1 Effect of *p*CMBS on PAH Transport by BL membrane vesicles

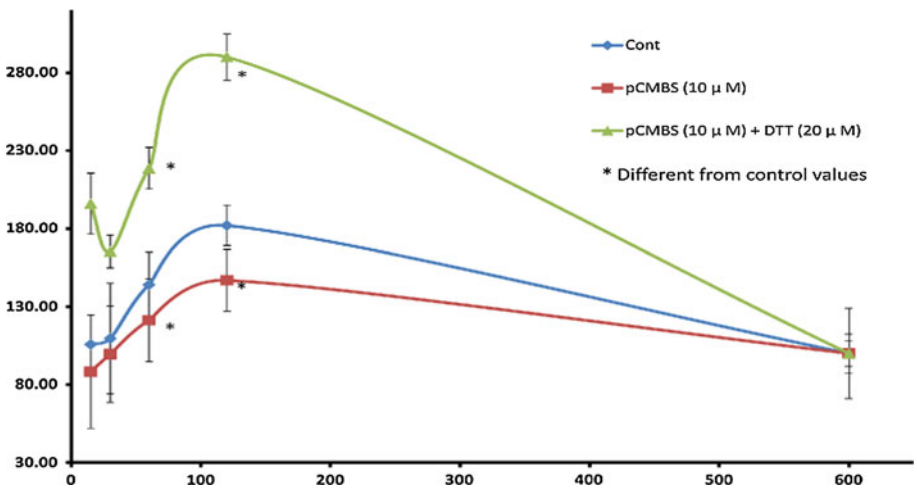

Fig. 2 Effect of DTT (20 μM) on *p*CMBS (10 μM) induced effect on PAH transport by BL membrane vesicles

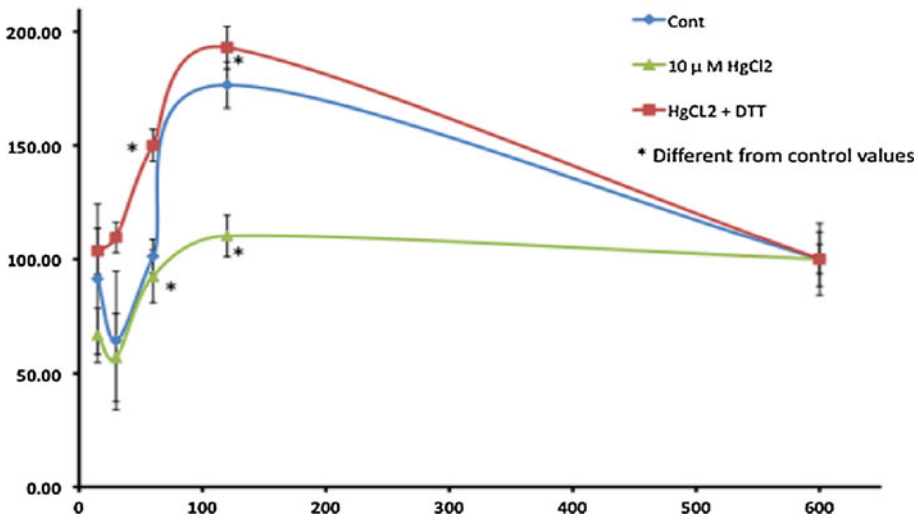

Fig. 3 Effect of DTT (20 μM) on $HgCl_2$ (5–10 μM) induced effect on PAH transport by BL membrane vesicles

decreased the overshoot value of the PAH transport by BL membrane vesicles (Figs. 1, 2, 3). Impairment of cellular redox status has been found to affect the transport of PAH by brush border membrane vesicles in ischemic acute renal failure (Montagna et al. 1998). In an attempt to recover the –SH groups of the proteins of membranes, *p*CMBS, and $HgCl_2$ (10 min) treated membranes were treated with DTT for 30 min and transport studies of PAH with BL membranes demonstrated the recovery of the transport process (Figs. 2, 3). The treatment of membrane vesicles with DTT, which were pretreated with *p*CMBS and $HgCl_2$, not only restored the transport overshoot values to control level but also further enhanced the values of overshoot over the control. The enhancement of overshoot values by DTT over the control values indicates that certain –SH groups may have been oxidized in membrane proteins during preparation which may have caused effects on transport process. Earlier, reduction in nonprotein sulfhydryls, i.e., GSH by diethylmaleate and buthionine sulfoximine (BSO) and enhancement of level of GSH by glutathione monethyl ester (GMEE) has been shown to affect the transport of PAH by BL membrane vesicles. The reduction of nonprotein sulfhydryls has shifted the overshoot of transport of PAH from 120 to 60 s. (Ansari et al. 1991). A detailed analysis of effects of DTT on membrane isolation and its effects on transport is required.

The transport of PAH occurs via organic anion transport system (Riedmaier et al. 2012). The human PAH transporter has long been cloned (Hosoyamada et al. 1999; Reid et al. 1998). Organic anion transporters from rat and other species have also been cloned and their function defined (Sekine et al. 1997; Sweet et al. 1997). Human organic anion transporter (hOAT1) has been found sensitive to sulfhydryls modifying agents (Astorga et al. 2011). A specific cystein (C440) mutation in between hOAT transporter is important for $HgCl_2$ sensitivity (Astorga et al. 2011). Similarly, mouse organic anion transporter 1 (mOAT1) has also been found sensitive to cysteine modification by *p*CMBS (Tanaka et al. 2004). Earlier, sulfhydryl reagents have been demonstrated to affect the PAH transport (Dantzler and Bentley 1983; Tse et al. 1983). The restoration of transport process by treatment with sulfhydryl reducing agents, like DTT after sulfhydryl modification with *p*CMBS, and $HgCl_2$ has not been demonstrated. In an earlier study, we demonstrated that effects of $HgCl_2$ on brush border mediated Na^+/glucose transport is observed by 16 h while recovery occurs by 48 h. This recovery is possible, probably, with the restoration of nonprotein sulfhydryls especially GSH, which may provide protection against sulfhydryl modification of membrane transporters (Ansari et al. 1990). The effects of these –SH modifying agents indicate that nephrotoxicant, such as $HgCl_2$ and possibly other toxicants producing oxidative stress in kidney epithelial cells may be acting after modifying the –SH groups of transporters and compromising the transport function of membrane. The study demonstrates that kidney PAH epithelial transport process can recover after restoration of sulfhydryl status of membrane transport systems which could be target of nephrotoxicants especially $HgCl_2$ and other xenobiotics which exhibit their toxicities by producing oxidative stress.

Acknowledgments Ansari, R.A. is grateful to Dr. William O. Berndt for his support of the study.

References

Ansari RA, Thakran RS, Berndt WO (1990) The effects of mercuric chloride on transport by brush border and basolateral membrane vesicles isolated from rat kidney. Toxicol Appl Pharmacol 106:145–153

Ansari RA, Thakran RS, Berndt WO (1991) Effects of mercuric chloride on renal plasma membrane function after depletion or

elevation of renal glutathione. Toxicol Appl Pharmacol 111:364–372

Astorga B, Wunz TM, Morales M, Wright SH, Pelis RM (2011) Differences in the substrate binding regions of renal organic anion transporters 1 (OAT1) and 3 (OAT3). Am J Physiol Renal Physiol 301:F378–F386

Baggett JM, Berndt WO (1986) The effect of depletion of nonprotein sulfhydryls by diethyl maleate plus buthionine sulfoximine on renal uptake of mercury in the rat. Toxicol Appl Pharmacol 83:556–562

Baker MA, Cerniglia GJ, Zaman A (1990) Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem 190:360–365

Boumendil-Podevin EF, Podevin RA (1983) Isolation of basolateral and brush-border membranes from the rabbit kidney cortex. Vesicle integrity and membrane sidedness of the basolateral fraction. Biochim Biophys Acta 735:86–94

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Dantzler WH, Bentley SK (1983) Effects of sulfhydryl reagent, *p*-chloromercuribenzoate, on *p*-aminohippurate transport by isolated, perfused snake renal tubules. Renal Physiol 6:209–217

Esteva-Font C, Ballarin J, Fernandez-Llama P (2012) Molecular biology of water and salt regulation in the kidney. Cell Mol Life Sci 69:683–695

Hori R, Takano M, Okano T, Inui K (1985) Transport of *p*-aminohippurate, tetraethylammonium and D-glucose in renal brush border membranes from rats with acute renal failure. J Pharmacol Exp Ther 233:776–781

Hosoyamada M, Sekine T, Kanai Y, Endou H (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. Am J Physiol 276:F122–F128

Houston MC (2011) Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. J Clin Hypertens (Greenwich) 13:621–627

Jensen RE, Berndt WO (1988) Epinephrine and norepinephrine enhance *p*-aminohippurate transport into basolateral membrane vesicles. J Pharmacol Exp Ther 244:543–549

Klonne DR, Johnson DR (1983) Amelioration of mercuric chloride-induced acute renal failure by dithiothreitol. Toxicol Appl Pharmacol 70:459–466

Lash LH (2011) Renal membrane transport of glutathione in toxicology and disease. Vet Pathol 48:408–419

Lee HY, Kim KR, Woo JS, Kim YK, Park YS (1990) Transport of organic compounds in renal plasma membrane vesicles of cadmium intoxicated rats. Kidney Int 37:727–735

Lenaz G (2012) Mitochondria and reactive oxygen species. Which role in physiology and pathology? Adv Exp Med Biol 942:93–136

McDowell EM, Nagle RB, Zalme RC, McNeil JS, Flamenbaum W, Trump BF (1976) Studies on the pathophysiology of acute renal failure. I. Correlation of ultrastructure and function in the proximal tubule of the rat following administration of mercuric chloride. Virchows Arch B Cell Pathol 22:173–196

Montagna G, Hofer CG, Torres AM (1998) Impairment of cellular redox status and membrane protein activities in kidneys from rats with ischemic acute renal failure. Biochim Biophys Acta 1407:99–108

Reid G, Wolff NA, Dautzenberg FM, Burckhardt G (1998) Cloning of a human renal *p*-aminohippurate transporter, hROAT1. Kidney Blood Press Res 21:233–237

Riedmaier AE, Nies AT, Schaeffeler E, Schwab M (2012) Organic anion transporters and their implications in pharmacotherapy. Pharmacol Rev 64:421–449

Scatena R (2012) Mitochondria and drugs. Adv Exp Med Biol 942:329–346

Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem 272:18526–18529

Sweet DH, Wolff NA, Pritchard JB (1997) Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. J Biol Chem 272:30088–30095

Tanaka K, Zhou F, Kuze K, You G (2004) Cysteine residues in the organic anion transporter mOAT1. Biochem J 380:283–287

Tse SS, Bildstein CL, Liu D, Mamelok RD (1983) Effects of divalent cations and sulfhydryl reagents on the *p*-aminohippurate (PAH) transporter of renal basal-lateral membranes. J Pharmacol Exp Ther 226:19–26

Zalme RC, McDowell EM, Nagle RB, McNeil JS, Flamenbaum W, Trump BF (1976) Studies on the pathophysiology of acute renal failure. II. A histochemical study of the proximal tubule of the rat following administration of mercuric chloride. Virchows Arch B Cell Pathol 22:197–216