Biochemical Pharmacology 85 (2013) 1379–1388

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Modulation of mitochondrial glutathione status and cellular energetics in primary cultures of proximal tubular cells from remnant kidney of uninephrectomized rats

Bavneet Benipal [1], Lawrence H. Lash *

Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201, United States

ARTICLE INFO

Article history:
Received 30 November 2012
Accepted 8 February 2013
Available online 16 February 2013

Keywords:
Uninephrectomy
Compensatory renal hypertrophy
Mitochondria
Oxidative stress
Glutathione

ABSTRACT

Compensatory renal hypertrophy following reduction in renal mass leads to a hypermetabolic state and increases in basal mitochondrial oxidative stress and susceptibility to several nephrotoxicants. Previous studies provide conflicting data on whether renal mitochondria after reduction in renal mass undergo proliferation or hypertrophy or both. In the present study, our goal was to determine whether mitochondria of hypertrophied kidney undergo hypertrophy or proliferation after uninephrectomy using the uninephrectomized (NPX) rat model. Renal proximal tubular (PT) cells from NPX rats exhibited increased mitochondrial density, membrane potential and protein but no significant difference in mitochondrial DNA, as compared to PT cells from control rats. Our previous studies showed that overexpression of two mitochondrial anion transporters, the dicarboxylate (DIC, *Slc25a10*) and oxoglutarate (OGC, *Slc25a11*) carriers, in NRK-52E cells resulted in increased mitochondrial uptake of glutathione (GSH) and protection from chemically induced apoptosis. In the present study, we overexpressed DIC- and OGC-cDNA plasmids to assess their function in renal PT cells after compensatory renal hypertrophy. PT cells from NPX rats that were first preincubated with GSH were protected from cytotoxicity due to the mitochondrial inhibitor antimycin A by overexpression of either of the two mitochondrial GSH transporters. Our present results provide further evidence that compensatory renal hypertrophy is associated primarily with mitochondrial hypertrophy and hyperpolarization and that manipulation of mitochondrial GSH transporters in PT cells of hypertrophied kidney can alter susceptibility to chemically induced injury under appropriate conditions and may be a suitable therapeutic approach.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Reductions in functional nephron mass, such as occur in the uninephrectomized (NPX) rat model, lead to compensatory renal hypertrophy and are associated with numerous biochemical, physiological and morphological changes in the remnant kidney [1–6]. There are also compensatory alterations in renal glutathione (GSH) content, such that the concentration of GSH within the renal cortex and outer stripe of the outer medulla and in isolated renal proximal tubular (PT) cells is increased on a per mg protein basis relative to that in renal tissue or cells from normal rats [6,7]. GSH is the most abundant non-protein thiol in mammalian cells and plays key roles in cells by functioning as an antioxidant, enhancing the immune system and in detoxification of reactive electrophiles.

Previous research showed that compensatory renal hypertrophy leads to an increase in energy demand and increased oxygen consumption per nephron [8–11], further leading to a hypermetabolic state. This suggests that alterations in mitochondrial function compensate for the greater need for energy in the hypertrophied kidney. In contrast to previous studies that concluded both renal mitochondrial proliferation and hypertrophy occurred [12,13], Hwang et al. [14] concluded that only mitochondrial hypertrophy of a fixed number of mitochondria

Abbreviations: AA, antimycin A; BSA, bovine serum albumin; tBH, tert-butyl hydroperoxide; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; DIC, dicarboxylate carrier; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSH, glutathione; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzyimidazolyl-carbocyanine iodide; KHB, Krebs-Henseleit buffer; LDH, lactate dehydrogenase; MVK, methyl vinyl ketone; NPX, uninephrectomized; NRK, normal rat kidney; OGC, 2-oxoglutarate carrier; PT, proximal tubular; RT-PCR, reverse transcription-polymerase chain reaction.

* Corresponding author at: Department of Pharmacology, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201, United States. Tel.: +1 313 577 0475; fax: +1 313 577 6739.

E-mail addresses: bbenipal@mail.med.upenn.edu (B. Benipal), l.h.lash@wayne.edu (L.H. Lash).

[1] Current address: University of Pennsylvania Medical Center, Institute for Environmental Medicine, A Center for Lung Biology and Baromedicine, John Morgan Building, 3620 Hamilton Walk, Philadelphia, PA 19104-6068, United States.

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.02.013

was associated with compensatory renal hypertrophy of PT cells. On the basis of these previous studies, which provided evidence for both renal mitochondrial proliferation and hypertrophy, we analyzed functions of renal mitochondria after compensatory renal hypertrophy due to NPX to assess the role of both proliferation and hypertrophy in development of the hypermetabolic state of PT cells from NPX rats.

To provide a mechanistic analysis of the biochemical effects of NPX, we previously studied the biochemical function and toxicant susceptibility of the remnant kidney in suspensions of freshly isolated PT cells from NPX rats [15,16]. The underlying foundation of this in vitro model is that biochemical and functional changes that occur in the renal PT cell after uninephrectomy and compensatory renal growth will be retained upon isolation of the cells. This model has been extensively used in biochemical toxicology studies and has the advantages of definition and manipulation of incubation conditions and direct comparison between cells from control and NPX rats [17,18]. This model was extended to use of primary cultures of renal PT cells from NPX rats [19,20], which were demonstrated to retain several of the properties and functions of the hypertrophied PT cell in the intact kidney, thus enabling study of additional processes over a longer period of time than is possible in suspensions of freshly isolated cells.

Mitochondria play an important role in maintaining redox balance and are known as primary intracellular sites for generation of reactive oxygen species. Previous studies showed decreased concentrations of mitochondrial GSH associated with acute cytotoxicity [21] and apoptosis [22] in renal mitochondria and renal PT cells. Thus, maintenance of adequate concentrations of GSH within the mitochondrial matrix is essential for regulation and proper function of many critical processes. Our previous studies showed increased GSH in hypertrophied kidney as well as enhanced susceptibility to nephrotoxicants [20] and increased basal mitochondrial oxidative stress [23], suggesting that the compensatory increase in renal GSH content is insufficient to deal with the oxidative stress that arises after nephrectomy.

There are two main pools of GSH in renal PT cells, one in the cytoplasm and one in mitochondria [24]. In cytoplasm, GSH is synthesized by two ATP-dependent reactions catalyzed by γ-glutamyl cysteine synthetase (also called glutamate-cysteine ligase) and GSH-synthetase. As the predominant, if not sole location of these enzymes catalyzing synthesis of GSH is the cytoplasm, the mitochondrial pool of GSH must derive by active transport from the cytoplasm or in exchange for another anion [24]. Two organic anion carriers of the mitochondrial inner membrane, the dicarboxylate carrier (DIC; *Slc25a10*) and 2-oxoglutarate carrier (OGC; *Slc25a11*), were identified as being responsible for most of the transport of cytoplasmic GSH into the renal mitochondrial matrix [25,26]. We subsequently demonstrated that overexpression of either the DIC [27] or the OGC [28] in a renal PT cell line, NRK-52E cells, enhanced mitochondrial GSH content and protected the cells from cytotoxicity from oxidants and mitochondrial toxicants. These data suggested a possibility that this approach could be used to protect hypertrophied kidney cells from the oxidative stress associated with compensatory renal hypertrophy by further increasing renal mitochondrial GSH content. Taken together, these data suggest that increases in expression and activity of mitochondrial GSH transporters may be an effective means to decrease susceptibility to mitochondrial toxicants.

Based on all these previous observations, the primary goal of this study was to further expand on earlier studies [12,14] of mitochondrial profiles and our previous studies [27,28] of modulating renal mitochondrial redox status in renal PT cells after compensatory renal hypertrophy due to nephrectomy. In the present study, we confirmed that compensatory renal hypertrophy leads primarily to renal mitochondrial hypertrophy and hyperpolarization rather than proliferation, further leading to increased mitochondrial basal oxidative stress, as shown in our recent work [23]. In addition, our data also provide evidence that manipulation of mitochondrial GSH transport can modulate the response to a mitochondrial toxicant under appropriate conditions, supporting the toxicological significance of the mitochondrial GSH transport process after compensatory renal hypertrophy.

2. Materials and methods

2.1. Chemicals

Percoll, collagenase (type I; EC 3.4.24.3), type I collagen, bovine serum albumin (BSA; fraction V), penicillin G, streptomycin, amphotericin B, insulin (from bovine pancreas), human transferrin, sodium selenite, hydrocortisone, epidermal growth factor (EGF; from mouse submaxillary glands), and 3,3′,5-triiodo-DL-thyronine (T3) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM/F-12, 50/50, 1×) was purchased from Mediatech Inc. (Herndon, VA, USA). Polystyrene tissue culture dishes were purchased from Corning Inc. (Corning, NY, USA). Polypropylene micromesh (210 μm pore size) was purchased from SpectraPor Inc. (Rancho Dominguez, CA, USA). All other chemicals were of the highest purity available and were purchased from commercial sources.

2.2. Surgical procedure

Male Sprague-Dawley rats (150–175 g body weight) were used in the present study. Animals that underwent surgical nephrectomy (removal of right kidney) were allowed a minimum 10-day recovery period prior to experiments, which has been established as the amount of time required for completion of the compensatory renal hypertrophy response [6]. Right-side nephrectomized (NPX) rats were purchased from Harlan (Indianapolis, IN, USA). The surgical procedure was performed as described previously [6]. Control rats were surgically naïve because previous studies showed that sham surgery has no apparent effect on the compensatory growth response [6,16,29]. Control and NPX rats were age-matched for all studies.

2.3. Primary culture of rat PT cells

Isolated renal cortical cells were obtained by collagenase perfusion [30]. PT cells were enriched from the cortical cells by Percoll density-gradient centrifugation [31] and the PT cells were then placed into primary culture [32]. A day before perfusion, all the glassware and surgical tools were sterilized. On the day of perfusion, Hank's concentrate (5×), containing 137 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4 \cdot 7H_2O$, 0.42 mM Na_2HPO_4, 0.44 mM KH_2PO_4 and 25 mM $NaHCO_3$, was used to make Hank's I (25 mM Hepes, 0.5 mM EGTA and 2%, w/v BSA) and Hank's II (4 mM $CaCl_2$ and 0.15%, w/v collagenase) buffers. Krebs-Henseleit buffer (KHB) Concentrate #1 (2×), containing 118 mM NaCl, 4.8 mM KCl, 0.96 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$ and 2.55 mM $CaCl_2 \cdot 2H_2O$, was used to make KHB-1 buffer. KHB Concentrate #2 (10×), containing 118 mM NaCl, 4.8 mM KCl, 0.96 mM KH_2PO_4, 0.12 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$ and 25 mM Hepes, was used to make KHB-2 buffer. All buffers were bubbled with 95% O_2/5% CO_2 for 30 min and adjusted to pH 7.4.

Download English Version:

https://daneshyari.com/en/article/5823510

Download Persian Version:

https://daneshyari.com/article/5823510

Daneshyari.com