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Biochemical and Functional Characteristics of Cultured Renal Epithelial Cells from Uninephrectomized Rats: Factors Influencing Nephrotoxicity

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ABSTRACT

Primary cultures of renal proximal (PT) and distal tubular (DT) cells from control and uninephrectomized (NPX) Sprague-Dawley rats were established to characterize factors that are responsible for the altered susceptibility to nephrotoxicants that occurs after compensatory renal cellular hypertrophy. Cells were grown in serum-free, hormonally defined medium and parameters were measured on days 1, 3, and 5 of primary culture. PT and DT cells from control and NPX rats appeared to maintain epithelial characteristics in culture, as shown by cytokeratin staining, morphology, protein and DNA content, and enzyme activities. Activities of several glutathione-dependent enzymes, including γ-glutamyltransferase, glutathione *S*-transferase, glutathione peroxidase, and γ-glutamylcysteine synthetase, were significantly greater in PT cells from NPX rats than in PT cells from control rats when factored by protein content. Rates of α-methylglucose uptake across the basolateral and brush-border membranes and sodium-dependent uptake of glutathione across the basolateral membrane were 2- to 3-fold higher in PT cells from NPX rats than in PT cells from control rats. These results are consistent with the hypertrophied phenotype being maintained in primary cultures of PT cells from NPX rats. The marked alterations in transport may play central roles in the delivery of nephrotoxicants to the target cell, and thus, increases the probability of chemically induced injury or death. These findings also suggest that these cell cultures may be useful for the study of biochemical processes associated with compensatory renal cellular hypertrophy.

Reduced functional renal mass is a relatively common condition in humans that results from a host of factors, such as renal disease, surgery, or aging. Within a short period of time after a significant number of functioning nephrons has been reduced, the remnant renal tissue undergoes profound morphological and functional changes by mechanisms that still remain unclear (Meyer et al., 1996). In rodents, the acute hemodynamic, functional, and biochemical effects of compensatory renal growth are nearly complete within 7 to 10 days after surgery (Zalups et al., 1987; Meyer et al., 1996). The cellular changes associated with uninephrectomy are most prominent in the proximal tubular (PT) region of the nephron (Meyer et al., 1996), and include cellular hypertrophy and increased cellular content of protein (Meyer et al., 1996), increased transport of sodium ions (Meyer et al., 1996), increased rates of mitochondrial electron transport (Harris et al., 1988), increased cellular synthesis and content of glutathione (GSH) and metallothionein (Zalups and Veltman, 1988; Zalups and Lash, 1990, 1994), and increased activities of several GSH-dependent enzymes (Lash and Zalups, 1994). There are toxicological implications of this hypertrophied state because rats that have undergone uninephrectomy and compensatory renal growth (NPX rats) exhibit altered susceptibility to various nephrotoxicants, including inorganic mercury (Zalups and Diamond, 1987; Zalups and Lash, 1994; Zalups, 2000), analgesics (Mollard, 1976; Henry et al., 1983), and cadmium-metallothionein (Zalups et al., 1992).

To study some of the biochemical properties of renal epithelial cells from NPX rats, we previously prepared suspensions of freshly isolated renal PT cells from NPX rats by collagenase perfusion and density-gradient centrifugation in Percoll, and showed that these cells retained their increased cell size, increased protein content, and increased activities of several enzymes (Lash and Zalups, 1992, 1994). We also prepared cells from the distal tubular (DT) region of the nephron to examine a nephron segment that is not the pri-

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ABBREVIATIONS: PT, proximal tubular; GSH, glutathione; NPX, uninephrectomized; DT, distal tubular; AMG, α-methylglucose; GDH, glutamate dehydrogenase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; GCS, γ-glutamylcysteine synthetase; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; BBM, brush-border membrane; BLM, basolateral membrane.

mary one exhibiting compensatory hypertrophy in vivo (Zalups et al., 1985).

Suspensions of freshly isolated cells provide a convenient model to study processes such as transport, metabolism, and acute cytotoxicity. However, the in vitro model is restricted to the study of short-term processes because of the limited time-period (up to 4 h) over which the cells remain viable. Hence, to study processes and responses that occur over longer periods (hours to days), it becomes necessary to develop an in vitro model that retains viable cellular function and the cellular phenotype found in the intact tissue over those periods. We have previously placed suspensions of freshly isolated PT and DT cells from the rat in primary culture, and demonstrated that they retain epithelial morphology, function, and biochemistry for at least 5 days (Lash et al., 1995). The primary cultures afford the opportunity to study processes and responses such as the influence of growth factors on cell growth and differentiation, expression of drug metabolism enzymes, and the effects of longer-term exposures to toxic chemicals on cellular function.

The present study was designed to test the hypothesis that the morphological, biochemical, and physiological changes that occur in the kidneys as a consequence of compensatory renal cellular hypertrophy are retained when isolated renal cells from both PT and DT regions are placed in primary culture. Validation of this in vitro model will enable the use of these primary cell cultures in the study of the biochemical and physiological processes that occur in the kidneys following a significant reduction in renal mass and characterization of factors that contribute to the known alterations in susceptibility of NPX rats to nephrotoxicants. This is apparently the first attempt to culture renal cells from kidneys that have undergone compensatory growth following uninephrectomy.

Experimental Procedures

Materials. Acivicin [L-(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid], Percoll, collagenase (type I), powdered 1:1 mixture of Dulbecco's modified Eagle's medium:Ham's F-12, HEPES, bovine serum albumin (fraction V), γ-glutamyl-*p*-nitroanilide, penicillin G, streptomycin sulfate, amphotericin B, insulin (from bovine pancreas), human transferrin, sodium selenite, hydrocortisone, 3,3′,5′-triiodo-DL-thyronine, and thyrocalcitonin (from bovine thyroid gland) were purchased from Sigma Chemical Co. (St. Louis, MO). Epidermal growth factor was purchased from Upstate Biotechnology (Lake Placid, NY). Polystyrene tissue culture dishes were purchased from Falcon (Becton Dickinson, Franklin Lakes, NJ) or Corning (Acton, MA) and Teflon cell scrapers were purchased from Falcon. α-[U-^{14}C]Methylglucose (AMG; specific activity 53 mCi/mmol) was purchased from NEN DuPont (Boston, MA).

Animals and Surgical Procedure. Male Sprague-Dawley rats (175–200 g; Harlan, Indianapolis, IN) were allowed access to food and water ad libitum and were kept in a room on a 12-h light/dark cycle. Animals that underwent surgical nephrectomy (removal of right kidney) were allowed a minimum 10-day recovery period before isolation of renal PT and DT cells. For uninephrectomy, each rat was anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg of body weight) before surgery. Uninephrectomy was performed by removal of the right kidney as described previously (Zalups and Lash, 1990). Control rats were surgically naïve, because previous studies have shown that sham surgery has no effect on the compensatory growth response (Zalups and Lash, 1990; Zalups, 1995; Lash et al., 1999).

Isolation of Rat Renal PT and DT Cells. Before surgery, all glassware and surgical tools were sterilized in an autoclave and the abdomen of the rat was shaved and cleansed with 70% (v/v) ethanol. Isolated renal cortical cells were obtained by collagenase perfusion (Jones et al., 1979; Lash and Tokarz, 1989). Briefly, kidneys (or the remnant kidney in NPX rats) were perfused first with EGTA-containing, Ca^{2+}-free Hanks' buffer at a flow rate of 8 ml/min for 10 min, followed by perfusion with Hanks' buffer containing 0.15% (w/v) collagenase (type I) and 2 mM $CaCl_2$ for 13 to 18 min at a flow rate of 5 ml/min. All buffers were continuously bubbled with 95% O_2, 5% CO_2 and maintained at 37°C. The collagenase used was approximately 300 units/mg of dry weight, with one unit defined by the supplier (Sigma Chemical Co.) as the amount of enzyme that will release peptides from native collagen and give the equivalent in ninhydrin color of 1.0 μmol of L-leucine in 5 h at pH 7.4 and 37°C in the presence of Ca^{2+} ions. At the conclusion of the collagenase perfusion, cells were released into Krebs-Henseleit buffer, pH 7.4, supplemented with 2.55 mM $CaCl_2$. 10 mM HEPES, and 2% (w/v) bovine serum albumin. Cell count and cell viability were estimated by mixing 0.1 ml of cells with 0.4 ml of 0.2% (w/v) trypan blue in saline and counting the total number of cells and cells that took up the dye on a hemacytometer. Typically, 85 to 95% of the cells from both control and NPX rats excluded the dye. Cell concentration, if necessary, was adjusted to between 5 and 8×10^6 cells/ml by dilution with Krebs-Henseleit buffer.

To obtain enriched fractions of renal PT and DT cells, cortical cells (5 ml, 5–8×10^6 cells/ml) were layered on 35 ml of an isosmotic 45% (v/v) Percoll solution in 50-ml polycarbonate centrifuge tubes and centrifuged for 30 min at 20,000*g* (Lash and Tokarz, 1989). The PT (upper layer) and DT (lower layer) cells were estimated to have purities of 97 and 88%, respectively, based on marker enzyme activities and cell type-specific respiratory responses (Lash and Tokarz, 1989; Lash, 1990). Based on enzymology and morphology (Lash and Tokarz, 1989; Lash, 1990), the renal PT cell preparation contains cells derived from both convoluted and straight segments of the proximal tubule; the renal DT cell preparation contains cells derived from the distal convoluted tubule, the cortical collecting duct, and the connecting tubule, but not from the medullary thick ascending limb, and is estimated to have less than 10% contamination from PT cells. Cell count and cell viability were estimated with trypan blue on a hemacytometer as described above. Cell viability (i.e., fraction of cells that excluded trypan blue) of both the PT and DT cells obtained after the Percoll separation from both control and NPX rats was typically 90 to 95%. Cell counts were not adjusted for viability before cells were plated for culture. For cell culture, freshly isolated PT and DT cells were suspended in 2 ml of Krebs-Henseleit buffer and diluted with an appropriate amount of culture media before plating.

Cell Culture Media. Basal medium was a 1:1 mixture of Dulbecco's modified Eagle's medium:Ham's F-12. Supplementation for both cell types included 15 mM HEPES, pH 7.4, 20 mM $NaHCO_3$, 5 μg of insulin/ml, 5 μg of human transferrin/ml, 100 ng of hydrocortisone/ml, 100 ng of epidermal growth factor/ml, 30 nM sodium selenite, and an antibiotic mixture containing 192 IU of penicillin G/ml, 200 μg of streptomycin sulfate/ml, and 2.5 μg of amphotericin B/ml. Other supplements included 7.5 pg of triiodothyronine/ml for PT cells and 5 ng of thyrocalcitonin/ml for DT cells. Optimization of cell culture media for primary cultures of rat PT and DT cells was described previously (Lash et al., 1995).

Primary Culture. PT and DT cells were seeded at a density of 0.2×10^6 cells/ml in media on 35-mm polystyrene tissue culture dishes that had been coated with a 0.1 mg/ml collagen solution (Vitrogen 100; Collagen Corp., Palo Alto, CA). In cultures used for transport studies, the cells were plated onto 30-mm, 4-μm pore size Millicell-PCF culture plate inserts (Millipore, Bedford, MA). Cultures were grown at 37°C in a humidified incubator under an atmosphere of 95% air, 5% CO_2. Fresh media were added to the dishes after 24 h (day 1) and every 48 h thereafter. Cells were harvested by

gently scraping the surface with a Teflon scraper and enzyme assays, and protein and DNA measurements were made on days 1, 3, and 5.

Protein, DNA, and Enzyme Assays. Protein contents were measured spectrophotometrically by the bicinchoninic acid method (Pierce, Rockford, IL) using bovine serum albumin as the standard. DNA content was measured spectrofluorometrically by complexing DNA with diamidinophenylindole according to Sorger and Germinario (1983) using calf thymus DNA as the standard. Glutamate dehydrogenase (GDH; EC 1.4.1.2) activity was measured as NADH oxidation in the presence of 2-oxoglutarate as described by Schmidt and Schmidt (1983). Lactate dehydrogenase activity (LDH; EC 1.1.1.27) was measured as NADH oxidation in the presence of pyruvate according to Kornberg (1955). γ-Glutamyltransferase (GGT; EC 2.3.2.2) activity was measured at 410 nm as *p*-nitroanilide formation with γ-glutamyl-*p*-nitroanilide and glycylglycine as substrates according to Orlowski and Meister (1963). γ-Glutamylcysteine synthetase activity (GCS; EC 6.3.2.2) was measured spectrophotometrically as NADPH oxidation in the presence of L-glutamate, ATP, phosphoenolpyruvate, and L-aminobutyrate as substrates according to Seelig and Meister (1984). Glutathione peroxidase activity (GPX; EC 1.11.1.19) was measured spectrophotometrically as the oxidation of NADPH in the presence of GSH, hydrogen peroxide, and glutathione reductase (Lawrence and Burk, 1976). Glutathione *S*-transferase activity (GST; 2.5.1.18) was measured spectrophotometrically at 340 nm as *S*-2,4-dinitrophenyl-GSH formation with 1-chloro-2,4-dinitrobenzene and GSH as substrates (Habig et al., 1974). (Na^+ + K^+)-stimulated ATPase (EC 3.6.1.37) activity was measured as the difference in NADH oxidation in the presence or absence of 0.1 mM sodium orthovanadate using phosphoenolpyruvate and ATP as substrates (Schmidt and Dubach, 1971).

Immunocytochemical Staining for Cytokeratins. Cytokeratins were monitored as an epithelial cell marker of renal PT and DT cells on day 5 of culture. Following fixation with 3.7% (v/v) formaldehyde and blocking with 0.2% (w/v) bovine serum albumin, cells were incubated with a monoclonal anti-pan cytokeratin antibody conjugated to fluorescein isothiocyanate in phosphate-buffered saline containing 0.1% (v/v) saponin. The stained cells were viewed and photographed with a Zeiss LSM 310 confocal laser scanning microscope.

Transport Studies. Cellular transport of GSH and [^{14}C]AMG was measured in PT and DT cells that had been seeded on 30-mm diameter, 0.4-μm Millicell-PCF culture plate inserts that were placed in 35-mm plastic culture dishes and grown for 3 days. Media containing 5 mM final concentration of substrate was added to either the upper cell surface or lower cell surface to study transport across the brush-border membrane (BBM) or basolateral membrane (BLM), respectively. Incubations were performed for 0.5, 1, 2, 3, 5, and 10 min in the presence of Na^+ ions or under Na^+-free conditions with choline chloride replacing Na^+ ions. Results are expressed as initial rates, calculated over the linear range of uptake, in the presence of Na^+ ions (=total uptake or Na^+-dependent + Na^+-independent uptake) and in the absence of Na^+ ions (=Na^+-independent uptake). Na^+-dependent uptake rates were then calculated as the difference between uptake in the presence and absence of Na^+ ions. For measurement of GSH uptake, cells were first pretreated with 0.25 mM acivicin for 15 min to inhibit GGT activity (Visarius et al., 1996; Lash and Putt, 1999). GSH transport was quantitated by high performance liquid chromatography analysis of cellular GSH content following the physical removal of cells from the polycarbonate membrane and derivatization of perchloric acid extracts of cells with 1-fluoro-2,4-dinitrobenzene and iodoacetate (Visarius et al., 1996). AMG transport was measured by removing the polycarbonate filter at the appropriate time points and quantitation of the incorporated radiolabel by liquid scintillation counting using a Beckman LS6000IC counter.

Data Analysis. Results are expressed as mean ± S.E. values of measurements from the indicated number of separate cell preparations. Enzyme activities were normalized to both cellular protein and cellular DNA, whereas transport activities were normalized only to cellular protein. Significant differences among selected mean values were first assessed by a one- or two-way ANOVA. When significant *F* values were obtained with ANOVA, the Fisher's protected least significant difference *t* test was performed to determine which mean values were significantly different from each other with two-tailed *P* values < 0.05 considered significant.

Results

Morphology and Growth of PT and DT Cells in Primary Culture. Expression of cytokeratins is a commonly used marker for epithelial cells. PT and DT cells from both control and NPX rats cultured for 5 days exhibited intense staining for cytokeratins (Fig. 1), providing evidence for retention of epithelial properties. Immunofluorescent staining of both PT and DT cells from NPX rats was more intense than that of cells from control rats. Overall cell size and nuclear size were noticeably larger in PT cells from NPX rats than in PT cells from control rats, consistent with retention of the cellular hypertrophy phenotype even after 5 days in culture.

Cell growth was quantified by measurement of DNA and protein content in dishes containing PT and DT cells cultured for 1, 3, and 5 days (Fig. 2). DNA content, expressed as micrograms of DNA per milliliter of cultured cells, with equal numbers of cells from control and NPX rats seeded on dishes at day 0, was significantly lower in day 3 and day 5 cultures of PT cells from NPX rats than in corresponding PT cells from control rats. In contrast, protein content of PT cells from day 1, day 3, and day 5 cultures was significantly higher in cells from NPX rats than in those from control rats. The increase in protein content coupled with a decrease in DNA content is consistent with cellular hypertrophy. Cultured DT cells from NPX rats, on the other hand, exhibited no significant changes in either DNA or protein content over the time period of culture.

Activities of Cellular Energetics and GSH-Dependent Enzymes. Activities of three enzymes associated with cellular energetics were measured in primary cultures of PT and DT cells from control and NPX rats (Figs. 3 and 4). The (Na^+ + K^+)-stimulated ATPase is the primary consumer of cellular ATP in renal PT cells (Soltoff, 1986) and has been shown to be markedly elevated in proximal tubules after uninephrectomy and compensatory renal growth (Meyer et al., 1996). Consistent with previous findings, (Na^+ + K^+)-ATPase activity was significantly higher in PT cells from NPX rats than in PT cells from control rats at day 1 and day 5 of culture when activity was normalized to cellular protein (44 and 56% higher, respectively; Fig. 3, top left). These elevations are particularly significant because activities were normalized to content of cellular protein, which was also higher in PT cells from NPX rats than in PT cells from control rats. In contrast, no significant differences in the activity of (Na^+ + K^+)-ATPase were detected between DT cells from NPX rats and DT cells from control rats (Fig. 3, top right). Enzyme activities were also normalized to content of cellular DNA (Fig. 4). The increases in activity of (Na^+ + K^+)-ATPase in PT cells from NPX rats relative to PT cells from control rats were markedly accentuated when activity was normalized to cellular DNA (2.5- to 7-fold increases; Fig. 4, top left). Minimal differences were also observed in activity of (Na^+ + K^+)-ATPase between DT cells from control and NPX rats

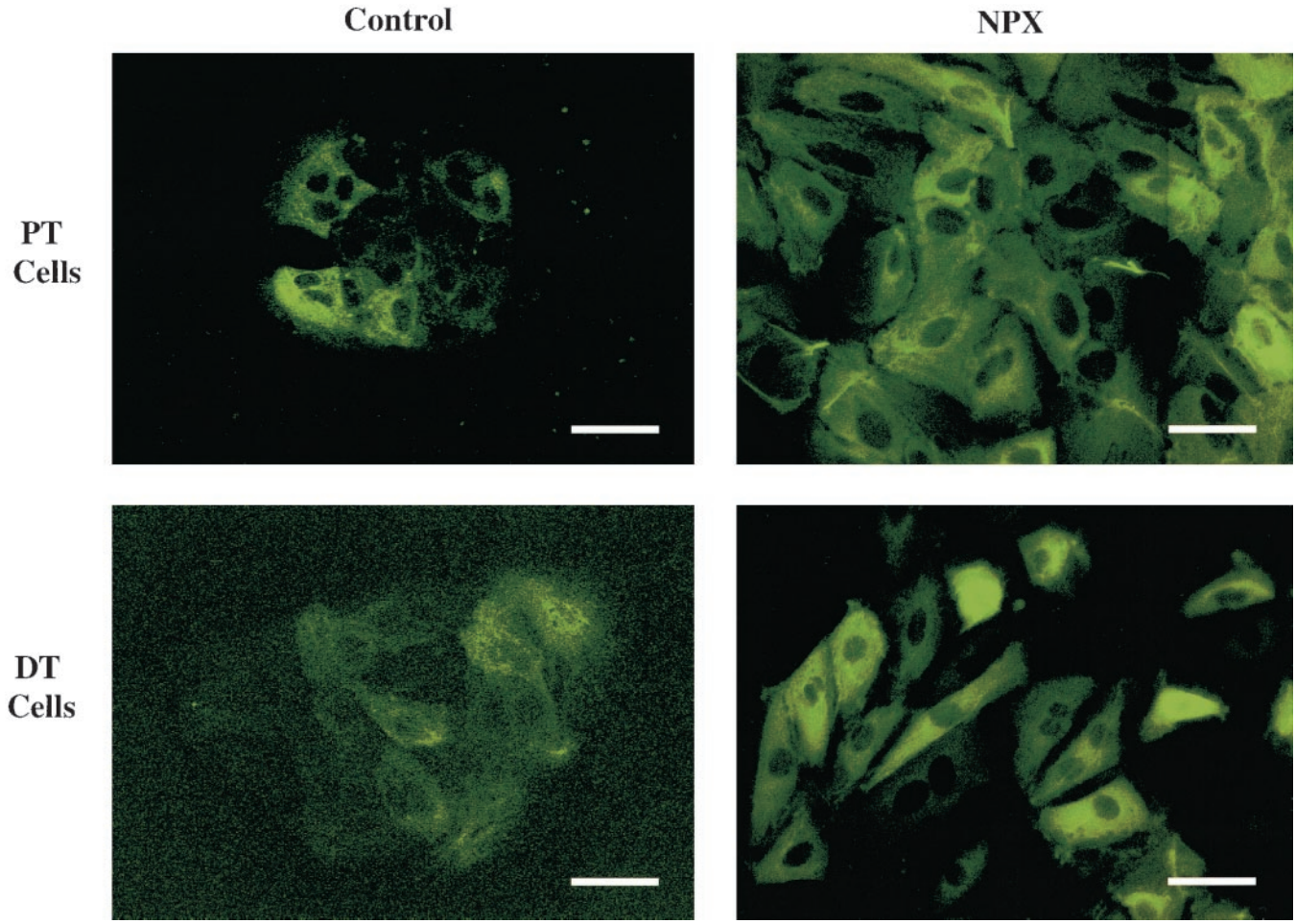

Fig. 1. Immunofluorescent staining for cytokeratins in renal PT and DT cells from control and NPX rats on day 5 of primary culture. Freshly isolated PT (top) and DT (bottom) cells from control (left) or NPX (right) rats were seeded at a density of 0.5 to 1.0 $\times$ 10^6 cells/ml and allowed to grow for 5 days. Cells were then washed twice with sterile phosphate-buffered saline and cytokeratin expression was visualized using a monoclonal fluorescein isothiocyanate-conjugated anti-mouse cytokeratin antibody. Note the enlarged size of the PT cells from the NPX rats relative to that of the PT cells from the control rats. Also note the greater intensity of cytokeratin staining in the cells isolated from the NPX animals relative to that in the corresponding cells from the control animals. Photomicrographs were taken at 100$\times$ magnification on a Zeiss confocal laser microscope. Bar, 5 μm.

when activity was normalized to cellular DNA (Fig. 4, top right).

The activity of GDH, which can be used as a marker for mitochondrial density, was 95 and 43% greater in PT cells from NPX rats than in PT cells from control rats at day 1 and day 3 of culture, respectively, when activity was normalized to cellular protein (Fig. 3, middle left). As with the activity of (Na^+ + K^+)-ATPase, no significant changes in the activity of GDH were detected between DT cells from NPX rats and DT cells from control rats (Fig. 3, middle right). Normalization of GDH activity to content of cellular DNA accentuated the increases in PT cells from NPX rats relative to PT cells from control rats (2.5- to 4-fold increases; Fig. 4, middle left), whereas there were still no significant differences in GDH activity between DT cells from control and NPX rats (Fig. 4, middle right).

LDH activity was significantly greater in PT cells from NPX rats than in PT cells from control rats only on day 1 of culture when activity was normalized to content of cellular protein (53% higher; Fig. 3, bottom left) but was markedly higher in PT cells from NPX rats than in PT cells from control rats on all 3 days when activity was normalized to content of cellular DNA (2.5- to 5.5-fold; Fig. 4, bottom left). No significant differences were observed in LDH activity between DT cells from control and NPX rats when activity was normalized to either cellular protein or DNA (Fig. 3, bottom right and Fig. 4, bottom right, respectively).

Activities of four GSH-dependent enzymes were compared in PT and DT cells from control and NPX rats cultured for 1, 3, and 5 days (Figs. 5–7). The activity of GGT, which is present primarily on the BBM of PT cells, was more than 2.5-fold greater in PT cells from NPX rats than in corresponding PT cells from control rats at all times studied when activity was normalized to cellular protein (Fig. 5, top left) and was 3.6- to 11.4-fold higher in PT cells from NPX rats than in PT cells from control rats when activity was normalized to cellular DNA (Fig. 6, top left). GCS activity was significantly elevated by 49 and 62% in PT cells from NPX rats relative to PT cells from control rats at day 1 and day 3 of primary culture, respectively, when activity was normalized to cellular protein (Fig. 5, bottom left). When GCS activity was normalized to cellular DNA, activity was significantly elevated by 2.4- to 6.9-fold in PT cells from NPX rats relative to PT cells from control rats at all 3 days of culture (Fig. 6, bottom left). These results are consistent with the findings of elevated GCS activity (Lash and Zalups, 1994)

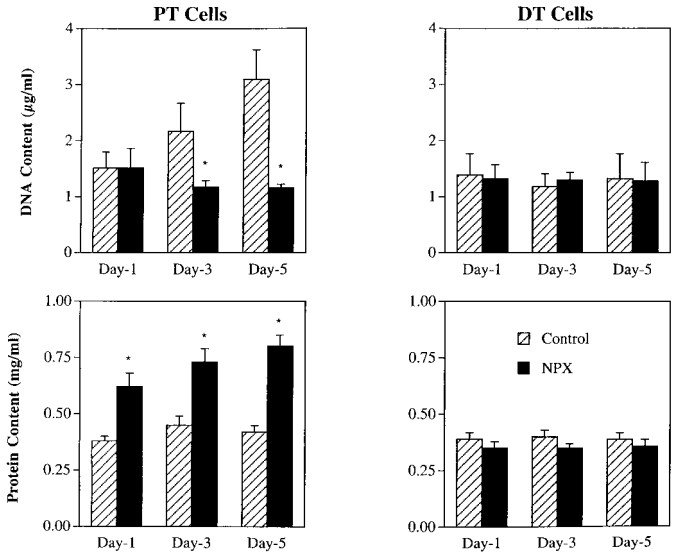

Fig. 2. DNA and protein contents in primary cultures of PT and DT cells from control and NPX rats. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and DNA or protein content was determined by colorimetric assays. Results are the means ± S.E.M. of measurements from 8 or 15 separate cell preparations, for DNA or protein measurements, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

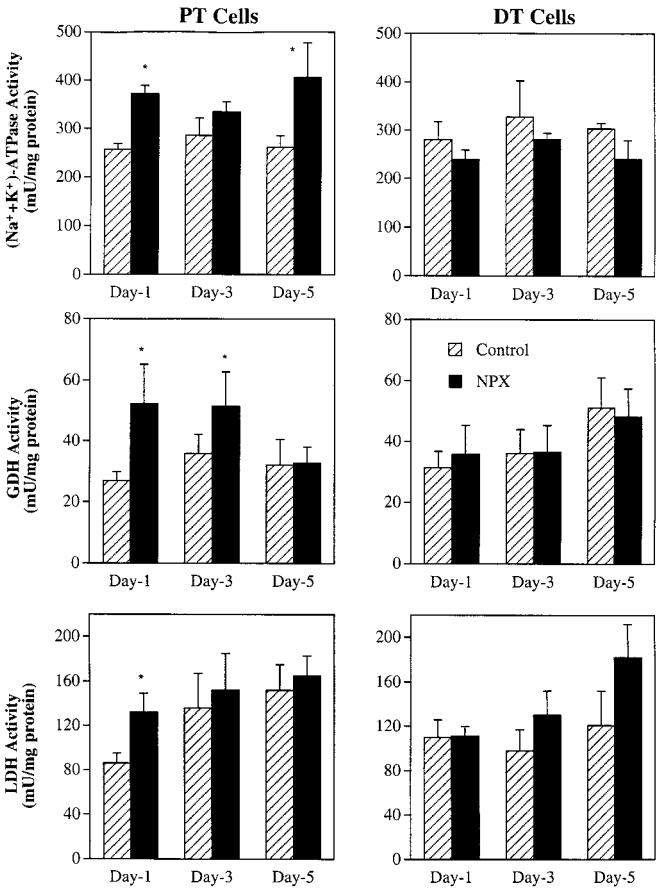

Fig. 3. Activities normalized to cellular protein of selected enzymes involved in cellular energetics in primary cultures of PT and DT cells from control and NPX rats. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of $(Na^+ + K^+)$-ATPase, GDH, and LDH were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from three, four to six, and four to seven separate cell preparations, for the ATPase, GDH, and LDH assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

and elevated cellular content of GSH in freshly isolated PT cells from NPX rats relative to those in PT cells from control rats (Lash and Zalups, 1992). GPX activity was more than 2-fold higher in PT cells from NPX rats than in PT cells from control rats at day 1, day 3, and day 5 of culture when activity was normalized to cellular protein (Fig. 7, top left) and was 3.4- to 14.2-fold higher in PT cells from NPX rats than in PT cells from control rats at day 1, day 3, and day 5 of culture when activity was normalized to cellular DNA (Fig. 8, top left). Similarly, GST activity with 1-chloro-2,4-dinitrobenzene as substrate was significantly elevated in PT cells from NPX rats at day 1 and day 3 of culture when activity was normalized to protein (68–110% higher; Fig. 7, bottom left) and these increases were accentuated when activity was normalized to cellular DNA (3.4- to 6.7-fold increases; Fig. 8, bottom left).

In contrast to the results in PT cells, GSH-dependent enzymes in DT cells were only modestly affected or decreased as a result of uninephrectomy, whether activities were normalized to either cellular protein or DNA (Figs. 5–8, right). GGT activity in DT cells was markedly lower than in PT cells, as was expected (Lash and Zalups, 1994, 1996) (it was only elevated in cells from NPX rats compared with that in cells from control rats at day 1 of culture). GCS activity was significantly greater in DT cells of NPX rats relative to that in DT cells from control rats only on day 1 of culture. Of the four enzyme activities measured, only GPX activity was consistently elevated over time in DT cells of NPX rats (relative to that in DT cells of control rats). In contrast, GST activity with 1-chloro-2,4-dinitrobenzene as substrate was decreased by as much as 65% in cells from NPX rats relative to that in control cells.

Plasma Membrane Transport of AMG and GSH. Both Na^+-dependent and Na^+-independent transport of glucose across BBMs and BLMs are characteristics of renal epithelial cells, and are typically measured with a nonmetabolizable analog of glucose such as AMG. By day 3 in culture, initial rates of both Na^+-dependent and Na^+-independent uptake of AMG, as well as total (i.e., Na^+-dependent + Na^+-independent) AMG uptake, across both the BBM and BLM were significantly greater in PT cells from NPX rats than in PT cells from control rats (Fig. 9A). Rates of transport of AMG across the BBM were approximately 3-fold greater in PT cells from NPX rats than in PT cells from control rats. At the BLM, the rates of transport in PT cells from NPX rats were approximately 2-fold greater than those in PT cells from control rats. Total transport of AMG across the BBM was slightly but significantly greater in DT cells from NPX rats than in DT cells from control rats (Fig. 9B). This increased transport could be accounted for solely by an increased Na^+-independent mechanism. In contrast, there were no significant differences in the rates of AMG transport across the BLM between DT cells from NPX rats and DT cells from control rats.

GSH is transported across the BBM of renal PT cells by a

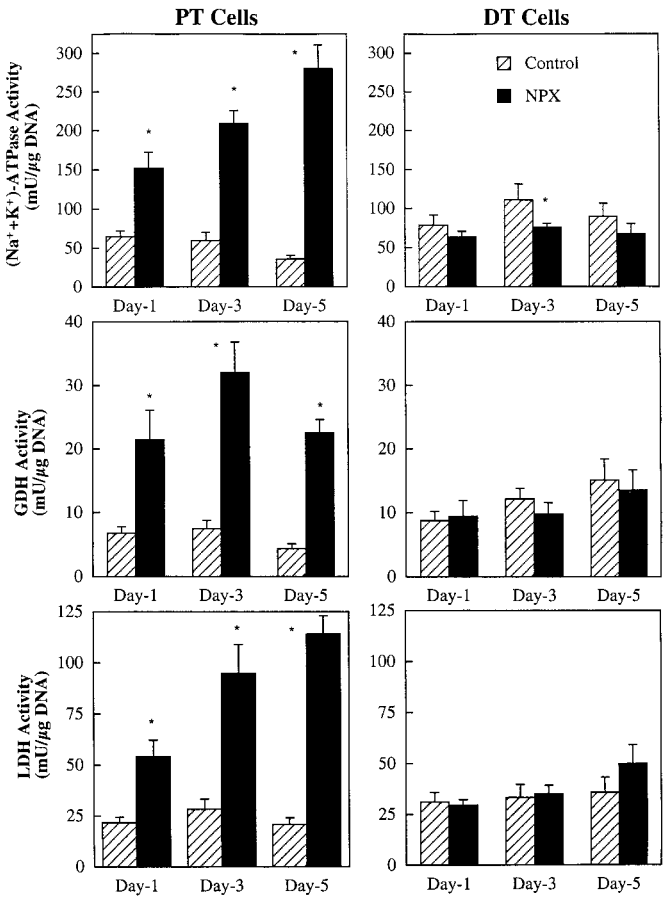

Fig. 4. Activities normalized to cellular DNA of selected enzymes involved in cellular energetics in primary cultures of PT and DT cells from control and NPX rats. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of ($Na^+ + K^+$)-ATPase, GDH, and LDH were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from three, four to six, and four to seven separate cell preparations, for the ATPase, GDH, and LDH assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

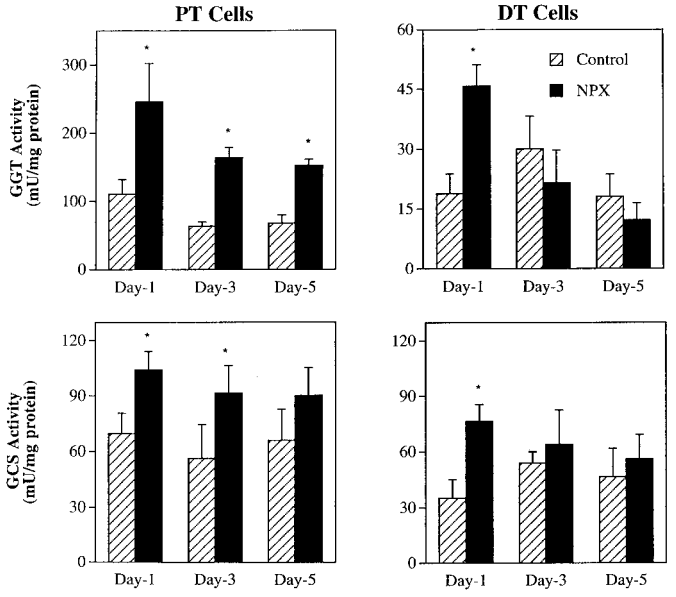

Fig. 5. Activities normalized to cellular protein of GSH-dependent enzymes in primary cultures of PT and DT cells from control and NPX rats: GGT and GCS. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of GGT and GCS were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from five to seven and six to nine separate cell preparations, for GGT and GCS assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

membrane potential-sensitive, Na^+-independent carrier (Inoue and Morino, 1985) and across the BLM of renal PT cells by Na^+-dependent and Na^+-independent carriers (Lash and Jones, 1984; Visarius et al., 1996; Lash and Putt, 1999). Although GSH transport occurs in DT cells, the rates are severalfold lower than those in PT cells (Lash and Putt, 1999). The cell type-specific patterns of GSH uptake were retained in primary cultures after 3 days of growth (Fig. 10). The total rates of GSH uptake across the BBM of PT cells from control rats were approximately twice those across the BBM of DT cells from control rats. Moreover, the total rates of GSH uptake across the BLM of PT cells from control rats were nearly 4-fold higher than those across the BLM of DT cells from control rats. In PT cells from both control and NPX rats, only Na^+-independent uptake was observed across the BBM. As a result of uninephrectomy, there was a 3-fold increase in Na^+-dependent and total GSH uptake across the BBM of PT cells and an approximate 3.5-fold increase in both Na^+-dependent and Na^+-independent rates of GSH uptake across the BLM of PT cells. DT cell cultures from NPX rats exhibited small but significant increases in rates of Na^+-

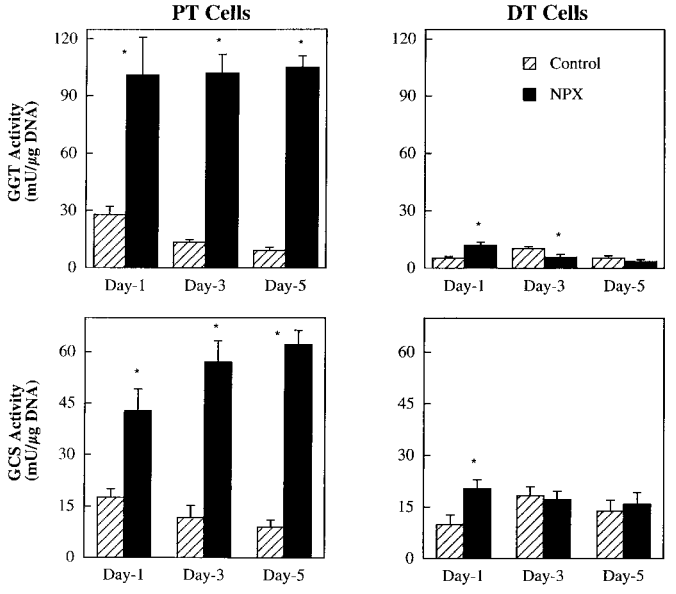

Fig. 6. Activities normalized to cellular DNA of GSH-dependent enzymes in primary cultures of PT and DT cells from control and NPX rats: GGT and GCS. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of GGT and GCS were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from five to seven and six to nine separate cell preparations, for GGT and GCS assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

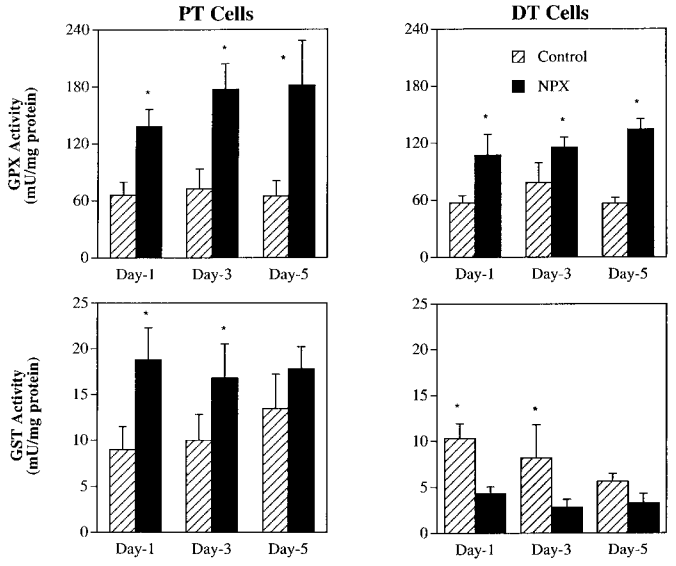

Fig. 7. Activities normalized to cellular protein of GSH-dependent enzymes in primary cultures of PT and DT cells from control and NPX rats: GPX and GST. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of GPX and GST were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from six and four to six separate cell preparations, for GPX and GCS assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

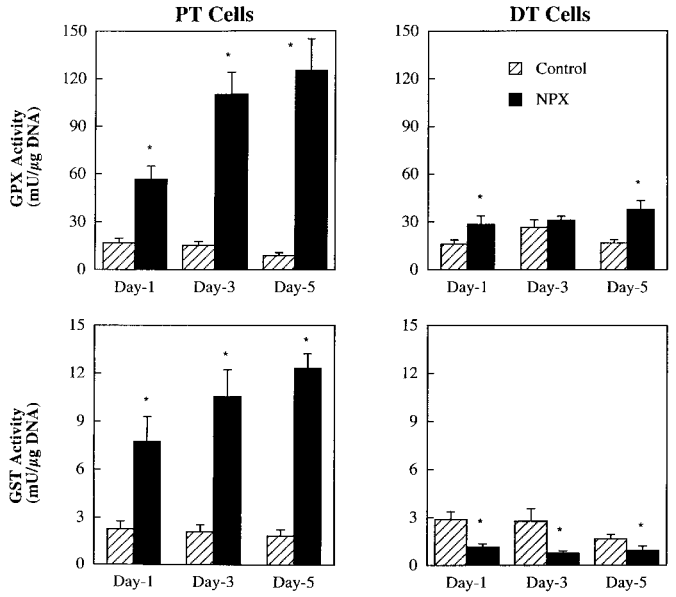

Fig. 8. Activities normalized to cellular DNA of GSH-dependent enzymes in primary cultures of PT and DT cells from control and NPX rats: GPX and GST. Freshly isolated PT or DT cells from control and NPX rats were seeded onto 35-mm polystyrene dishes at a density of 1.0×10^6 cells/ml. Cells were grown for 1, 3, or 5 days in serum-free, hormonally defined medium and activities of GPX and GST were determined by spectrophotometric assays. Results are the means ± S.E.M. of measurements from six and four to six separate cell preparations, for GPX and GCS assays, respectively. *, significantly different ($P < 0.05$) from the value in the corresponding control cells.

dependent uptake of GSH across both the BBM and BLM. However, no changes were observed in either Na^+-independent or total rates of GSH uptake across either membrane between the two populations of DT cells.

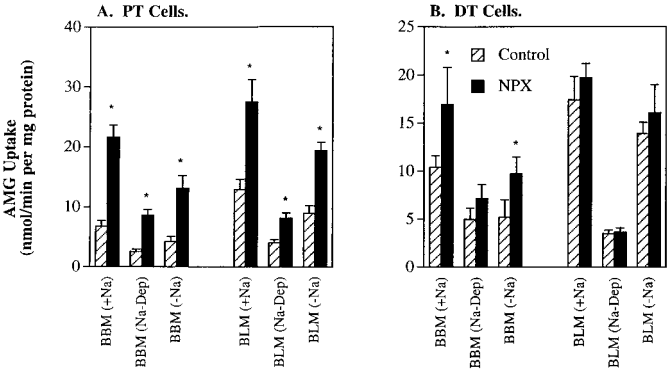

Fig. 9. Uptake of AMG in primary cultures of renal PT and DT cells from control and NPX rats. Cellular transport of 5 mM [^{14}C]AMG was measured in PT and DT cells that had been seeded on 30-mm diameter, 0.4-μm Millicell-PCF culture plate inserts that were placed in 35-mm plastic culture dishes and grown for 3 days. Media containing the appropriate concentration of substrate were added to either the upper cell surface or lower cell surface to study transport across the BBM or BLM, respectively. Incubations were performed for 0.5, 1, 2, 3, 5, and 10 min in the presence of Na^+ ions or under Na^+-free conditions with choline chloride replacing Na^+ ions. The Na^+-dependent uptake was calculated as the difference between uptake in the presence and absence of Na^+ ions. Results are expressed as initial rates, calculated over the linear range of uptake, and are the means ± S.E. of measurements from four separate cell cultures. AMG transport was measured by removing the polycarbonate filter at the appropriate time points and quantitation of the incorporated radiolabel by liquid scintillation counting using a Beckman LS6000IC counter. *, significantly different ($P < 0.05$) from the corresponding rate in cells from control rats.

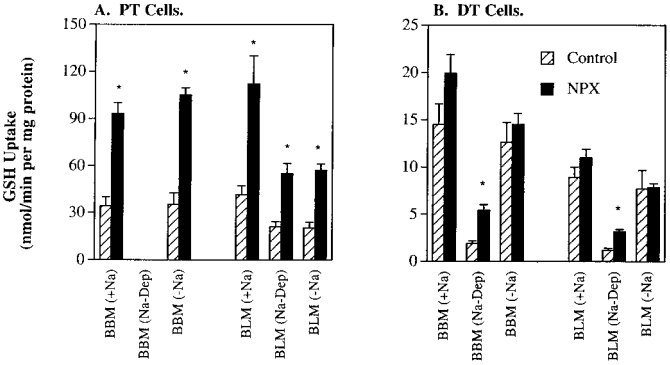

Fig. 10. Uptake of GSH in primary cultures of renal PT and DT cells from control and NPX rats. Cellular transport of 5 mM GSH was measured in PT and DT cells that had been seeded on 30-mm diameter, 0.4-μm Millicell-PCF culture plate inserts that were placed in 35-mm plastic culture dishes and grown for 3 days. Cells were pretreated with 0.25 mM acivicin for 15 min before initiation of transport measurements to inhibit GSH degradation. Media containing 5 mM final concentration of substrate were added to either the upper cell surface or lower cell surface to study transport across the BBM or BLM, respectively. Incubations were performed for 0.5, 1, 2, 3, 5, and 10 min in the presence of Na^+ ions or under Na^+-free conditions with choline chloride replacing Na^+ ions. The Na^+-dependent uptake was calculated as the difference between uptake in the presence and absence of Na^+ ions. Results are expressed as initial rates, calculated over the linear range of uptake, and are means ± S.E. of measurements from four separate cell cultures. GSH transport was quantitated by high performance liquid chromatography analysis of cellular GSH content following the physical removal of cells from the polycarbonate membranes and derivatization of perchloric acid extracts of cells with 1-fluoro-2,4-dinitrobenzene and iodoacetate. *, significantly different ($P < 0.05$) from the corresponding rate in cells from control rats.

Discussion

Compensatory renal cellular hypertrophy, which occurs after uninephrectomy, includes numerous biochemical and physiological changes that are most prominent in the PT

region of the nephron. Besides increases in cellular size and protein content, activities of various enzymatic and transport processes increase, even when these are normalized to the increased content of cellular protein. These increases are particularly significant and may be interpreted as an adaptive response of the hypertrophied cell to its altered physiological state. Parameters that increase over and above the increased content of cellular protein and that are likely to be part of the adaptive response include cellular GSH content (Zalups and Veltman, 1988; Zalups and Lash, 1990; Lash and Zalups, 1992), which is likely attributed to increased activity of GCS (Lash and Zalups, 1994), the rate-limiting enzyme in GSH biosynthesis, and activities of enzymes of the GSH redox cycle (Lash and Zalups, 1994). Additionally, the number of mitochondria per cell, and hence, the activity of mitochondrial electron transport, increase (Harris et al., 1988).

We have previously studied the effects of compensatory renal cellular hypertrophy on GSH metabolism and acute cellular injury induced by inorganic mercury, using freshly isolated renal cells prepared from control and NPX rats (Lash and Zalups, 1992, 1994; Lash et al., 1999). Although the suspensions of freshly isolated renal cells maintain expression of the hypertrophied phenotype, their applicability toward the study of biochemical, physiological, and toxicological effects of compensatory cellular hypertrophy is restricted to the study of short-term processes, due to the limited time period during which the cells remain viable. Suspensions of freshly isolated renal cells retain viability for at most 4 h (Lash and Tokarz, 1989; Lash, 1996). This time limitation has been addressed by the placement of cells or tissue in primary culture (Lash et al., 1995). The advantage of primary culture, compared with the use of established cell lines, is that primary cell cultures presumably reflect more accurately the properties of the in vivo tissue from which the cells were derived, although they may dedifferentiate to some degree during the culture period. Established cell lines are immortalized and often reflect only some of the properties of the in vivo cell type from which they were derived. Hence, the goals of the present work were to establish primary cultures of PT and DT cells from both control and NPX rats and to assess selected properties of basic cellular function to validate the cell cultures for their use in the study of biochemical, physiological, and toxicological effects of compensatory renal cellular hypertrophy. To our knowledge, this is the first time that renal cells from a hypertrophied kidney have been grown in culture and studied.

Examination of cytokeratin staining at day 5 of primary culture showed maintenance of expression of cytokeratins in cells from NPX rats, suggesting that epithelial properties were retained in these cells. However, differences were noted in PT and DT cells between control and NPX rats. Renal PT cells from NPX rats were noticeably larger than those from control rats at day 5 of culture. Consistent with the PT region of the nephron being the predominant region that exhibits the compensatory cellular hypertrophic response, primary cultures of DT cells from NPX rats did not exhibit the same obvious increase in cell size as did the cultures of PT cells. A detailed morphometric analysis of cell cultures from control and NPX rats will be necessary to confirm and elaborate on this conclusion.

Renal cellular hypertrophy of PT cells from NPX rats was corroborated by demonstration of increased cellular contents of protein and decreased cellular contents of DNA compared with values in the corresponding cultures from control rats. The absence of similar changes in contents of DNA and protein in DT cell cultures from NPX rats is consistent with the PT region of the nephron (as opposed to other nephron segments) being the predominant region that exhibits the compensatory hypertrophic response to reduced nephron mass. It is unlikely that the decrease in DNA content per milliliter of cell culture is due to inhibition of cell growth because protein content increased. Rather, we suggest that the decrease is due to the presence of a smaller number of larger, hypertrophied cells (each with presumably the same amount of DNA) taking up the available space on the culture dishes by day 3 and day 5 of culture.

The selective increases in activities of $(Na^+ + K^+)$-ATPase and GDH in primary cultures of PT cells from NPX rats are consistent with previous results obtained in freshly isolated PT cells (Lash and Zalups, 1994), and suggest that overall mitochondrial activity is enhanced in the PT cells as a consequence of compensatory cellular hypertrophy. Some of the elevated enzyme activities in PT cells from NPX rats decreased back toward control levels at later days of culture only when activities were normalized to cellular protein, suggesting that the cells may be gradually losing the hypertrophied phenotype during the course of cell culture. The diminution of the hypertrophic response, particularly by day 5 of culture, was by no means uniform, because activities of some enzymes [e.g., GGT, $(Na^+ + K^+)$-ATPase] remained significantly higher in PT cells from NPX rats than in PT cells from control rats, both when activities were normalized to cellular protein and DNA. Nonetheless, the general trend does appear to be for a modest diminution of the hypertrophic response with time in culture, suggesting that the cell culture model may be used best only up to day 3 of culture.

The potential significance of the enhanced mitochondrial function, which includes increases in mitochondrial density, rates of oxidative metabolism, and respiratory activity, is far-reaching and has implications for regulation of cellular redox status and susceptibility of renal PT cells to oxidative stress. Freshly isolated renal PT cells from the rat are sensitive to cellular injury from several oxidants (Lash and Tokarz, 1990). Renal PT cells, however, possess relatively high concentrations of GSH, activities of GSH-dependent enzymes, and the ability to transport extracellular GSH into the cell and protect against oxidative injury (Hagen et al., 1988; Lash and Tokarz, 1990; Lash and Zalups, 1994; Visarius et al., 1996; Lash and Putt, 1999). We have demonstrated previously that renal PT cells have higher concentrations of certain antioxidants, such as GSH, and elevated activities of several antioxidant enzymes than renal DT cells. Consequently, PT cells would appear to be more resistant to oxidative injury than DT cells. PT cells, but not DT cells, cultured from NPX rats were able to transport both AMG and GSH across both the BBM and BLM at much greater rates (50 to 300% increases) than the PT cells cultured from control rats. The increased transport activities are consistent with increased rates of intermediary and oxidative metabolism in the cells. Although the experimental design does not exclude the possibility that some of the increased rates of transport observed in NPX cells are due to paracellular leak, it is important to note that all measurements were done in parallel in cells from control and NPX rats under identical ex-

perimental conditions with cells grown on filter inserts. Basal LDH leakage values in both control and NPX cell cultures are similarly low (i.e., <10%) (data not shown), suggesting that there is no significant difference in membrane permeability of cells from the two surgical groups.

An important implication of the hypermetabolic state of PT cells from NPX rats is that their susceptibility to oxidative stress may be increased relative to cells from control rats. This is because the mitochondria are the major sites of oxygen consumption in the cell and they may produce partially reduced, reactive oxygen species, particularly when respiratory activity is increased. The increased activities of GCS, GPX, and GST that were observed in primary cultures of PT cells from NPX rats relative to activities in PT cells from control rats suggest an adaptive response of the hypertrophied cells to increased basal oxidative stress. The toxicological consequences of these changes in mitochondrial oxidative metabolism and in GSH-dependent metabolism and GSH transport will require additional study of the susceptibility of these cells to oxidants.

In conclusion, our studies are the first to establish primary cultures of PT cells from NPX rats as an in vitro model to study the biochemical and physiological processes and responses associated with compensatory renal cellular hypertrophy. Morphology, as observed microscopically, and cellular contents of protein and DNA, confirmed the retention of the hypertrophied phenotype through 5 days of primary culture. Consistent with the PT region of the nephron being the one that is most responsive to uninephrectomy, primary cultures of DT cells did not exhibit most of the changes in cellular morphology and enzyme activities that were observed in PT cells. Significant elevations in $(Na^+ + K^+)$-ATPase and GDH activities and in AMG transport relative to protein provide evidence of a hypermetabolic state in the hypertrophied cells. Significant elevations in activities of several GSH-dependent enzymes and in cellular GSH transport rates relative to protein are consistent with an adaptive response of the hypertrophied cells that may be associated with an increased basal oxidative stress.

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