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Activities of Enzymes Involved in Renal Cellular Glutathione Metabolism after Uninephrectomy in the Rat[1]

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The renal concentration of GSH increases after a significant reduction in renal mass and compensatory renal growth. To test the hypothesis that this increase is due to induction of GSH synthesis, the activities of γ-glutamylcysteine synthetase, other GSH-dependent enzymes, and selected enzymes involved in cellular energetics were measured in freshly isolated proximal tubular (PT) and distal tubular (DT) cells from male Sprague–Dawley rats that underwent uninephrectomy and compensatory renal growth or from sham-operated rats. Significant increases in cellular content of protein without increases in intracellular content of DNA, in both PT and DT cells, confirmed that cellular hypertrophy had occurred. γ-Glutamylcysteine synthetase activity increased significantly in PT cells, but not in DT cells, as a result of compensatory cellular hypertrophy, indicating that the effects of cellular hypertrophy on GSH synthesis occurred exclusively in the proximal tubule. Hypertrophy in PT cells, but not in DT cells, was associated with significant increases in activities of glutathione disulfide reductase, both Mg^{2+}-dependent and $(Na^+ + K^+)$-stimulated ATPases, succinate:cytochrome c oxidoreductase, and lactate dehydrogenase. Results from this study demonstrate that compensatory hypertrophy occurs in both PT and DT cells and that effects on GSH metabolism and cellular energetics associated with compensatory hypertrophy are more pronounced in PT cells than in DT cells. The findings also support our hypothesis that GSH synthesis is induced in the proximal tubule during compensatory hypertrophy. The increase in GSH synthesis may be an adaptive response to protect against oxidative stress caused by increases in mitochondrial metabolism.

The mammalian kidney undergoes compensatory growth after there has been a significant reduction in the number of functioning nephrons (see Refs. 1 and 2 for recent reviews). This growth is due mainly to cellular hypertrophy, rather than cellular hyperplasia. Some characteristics of hypertrophy that have been documented in proximal tubules (3, 4) and cortical collecting ducts (4–7) after renal mass has been reduced significantly include increases in cellular volume and increases in the area and surface density of the basolateral and luminal membranes. In addition to cellular hypertrophy, there are a number of physiological and biochemical changes that occur during compensatory renal growth, some of which are directly associated with cellular hypertrophy. Among the physiological changes that occur are increased renal blood flow, increased glomerular filtration rate, and increased water and electrolyte transport along segments of the remaining functional nephrons (1, 2–9). Biochemical changes that occur as a consequence of compensatory renal growth are less well characterized, although there are several that are known to occur invariably. These biochemical changes include increased Na^+/H^+ antiport activity in the proximal tubule (10), increased $(Na^+ + K^+)$-stimulated ATPase activity in segments of proximal and distal tubules (11–14), and increased mitochondrial metabolism along proximal tubules and in distal segments of the nephron (15, 16).

The changes that occur after renal mass is reduced significantly are mechanisms by which the remnant tissue adapts to lost renal function. Moreover, this adaptation occurs in part, as a response to increased work load imposed on the epithelial cells along each of the remaining nephrons. The hypermetabolic state that occurs in tubular epithelial cells, particularly in proximal tubular (PT)[3]

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[3] Abbreviations used: PT, proximal tubular; DT, distal tubular; NPX, uninephrectomized; SHAM, sham-operated; LDH, lactate dehydrogenase.

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cells, may cause increased chemical and mechanical stress that may eventually lead to renal failure (15–17). Furthermore, some of the adaptive responses associated with compensatory renal growth may increase susceptibility to certain forms of chemically or pathologically induced injury. As an example, uninephrectomized rats (NPX) in which compensatory renal growth occurs have a propensity to develop a more severe form of the nephropathy induced by mercuric chloride than sham-operated (SHAM) rats (18–22). The increased severity in renal injury is preceded by enhanced accumulation of inorganic mercury in pars recta segments of the proximal tubule in the outer stripe of the outer medulla (21, 23), which is where the toxic effects of inorganic mercury are primarily manifested.

We have shown previously that the renal concentration of GSH increases significantly after unilateral nephrectomy and compensatory renal growth, particularly in the outer stripe of the outer medulla (22, 24). Based on this observation and the known regulation of GSH biosynthesis by feedback regulation, we have hypothesized that compensatory renal growth causes increases in the intracellular concentrations of GSH by inducing the synthesis of γ-glutamylcysteine synthetase (EC 6.3.2.2), which is the rate-limiting enzyme involved in GSH biosynthesis. The hypermetabolism that results from compensatory renal growth may generate an oxidative stress, and the increase in renal GSH concentrations that we observed may be an adaptive response of the hypertrophied renal tubular epithelial cells to this stress.

In a subsequent study (25), we established an *in vitro* model system to study compensatory renal growth and chemical toxicity further. We employed freshly isolated PT cells from rat kidney and showed that the compensatory growth response was retained and expressed biochemically *in vitro*. Furthermore, when studied in the absence of bovine serum albumin in the medium, mercuric chloride was more cytotoxic in PT cells from NPX rats than from SHAM rats, thus reproducing the *in vivo* response (18–22).

In the current work, we employed freshly isolated PT cells and distal tubular (DT) cells from SHAM rats and from the remnant kidney of NPX rats to characterize further the mechanism of increase in renal GSH that occurs during compensatory renal cellular hypertrophy. The renal PT cell preparation contains cells derived from both convoluted and straight segments and is estimated to be at least 97% pure; the renal DT cell preparation contains cells derived from the distal convoluted tubule and the cortical collecting duct and connecting tubules, but not from the thick ascending limb, and is estimated to be 88% free of PT cell contamination (26). Activities of enzymes involved in GSH synthesis, GSH-dependent drug metabolism, and cellular energetics were measured in both renal PT cells and DT cells. Although PT cells are the most prominent site of physiological and biochemical changes during compensatory hypertrophy *in vivo*, other regions of the nephron also exhibit changes due to compensatory hypertrophy (4–8).

The results of the present study demonstrate that γ-glutamylcysteine synthetase activity was significantly higher in PT cells from NPX rats than in PT cells from SHAM rats, both at the level of the individual cell and when activity is expressed per milligram of protein. In contrast, γ-glutamylcysteine synthetase activity was not significantly altered in DT cells after compensatory cellular hypertrophy. However, biochemical indicators of compensatory hypertrophy, such as increased cellular protein and increased activities of certain enzymes, were observed in DT cells, confirming that this region of the nephron also undergoes changes as a consequence of reduced nephron number. A preliminary report of these findings has been presented (27).

EXPERIMENTAL PROCEDURES

Materials

Percoll, collagenase type I, diamidinophenylindole, calf thymus double-stranded DNA, pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (LDH; EC 1.1.1.27), L-α-aminobutyrate, γ-glutamyl-*p*-nitroanilide, and *p*-nitrophenylphosphate were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity available and were obtained from commercial sources.

Surgical Procedures

Male Sprague–Dawley rats (175–200 g at time of surgery; Harlan Sprague–Dawley, Indianapolis, IN) were used. Animals were housed in a room on a 12-h light–dark cycle in the Wayne State University vivarium. They were allowed access to a commercial laboratory diet for rats and water *ad libitum*. The rats were divided into two surgical groups, one that underwent uninephrectomy (NPX rats) and one that underwent a sham operation (SHAM rats). Animals were anesthetized with a single ip injection of sodium pentobarbital (50 mg/kg body wt) before surgery. Uninephrectomies were performed by removal of the right kidney as described previously (22). For the sham operations, the same surgical procedures were performed, except that the right renal blood vessels and ureter were not ligated and the right kidney was not excised.

Isolation of Rat Renal PT and DT Cells

Isolated renal cortical cells were obtained by collagenase perfusion (28). Prior to surgery, rats were anesthetized with a single ip injection of sodium pentobarbital (50 mg/kg body wt). To obtain enriched populations of PT and DT cells, cortical cells were subjected to Percoll density-gradient centrifugation as described previously (25, 26). Briefly, cortical cells (5 ml, 5 to 8×10^6 cells/ml) were layered on 35 ml of 45% (v/v) isosmotic Percoll solution in 50-ml polycarbonate centrifuge tubes and were centrifuged at 4°C for 30 min at 20,000*g* in a Sorvall RC2B centrifuge in an SS34 rotor. Fractions were collected and cell types of origin were determined by the use of marker enzymes and cell type-specific respiratory responses (26, 29). In previous studies (25), we showed that although renal cells from NPX rats undergo compensatory hypertrophy, the distribution of renal PT and DT cells on the Percoll gradient does not change significantly. Based on enzymology and morphology (26), the renal PT cell preparation contains cells derived from both convoluted and straight segments and is estimated to be at least 97% pure; the renal DT cell preparation contains cells derived from the distal convoluted tubule and the cortical collecting duct and connecting tubules,

but not from the thick ascending limb. Enzyme activities in the freshly isolated cells are not altered by the isolation procedure and, based on comparisons with activities in tissue homogenates, appear to reflect levels observed *in vivo* (25, 26, 28–30).

Before incubations, cells were diluted fivefold with Krebs–Henseleit buffer, pH 7.4, containing 25 mM Hepes, 2% (w/v) bovine serum albumin, and metabolic substrates (5 mM glucose, 5 mM glutamine), then washed to remove Percoll, and then resuspended in fresh buffer at concentrations of 2 to 6 $\times$ 10^6 cells/ml. Cell concentrations were determined in the presence of 0.2% (w/v) trypan blue in a hemacytometer, and cell viability was estimated by measuring the release of LDH activity from the cells in the presence and absence of detergent (26). All buffers were equilibrated with 95% O_2/5% CO_2 and incubations were performed at 37°C under an atmosphere of 95% O_2/5% CO_2 in a Dubnoff metabolic shaking incubator (60 cycles/min).

Experimental Protocol

Isolated renal cells were obtained from two groups with no other treatment, one derived from SHAM rats (n = 6) and one derived from NPX rats (n = 7). Cells were isolated from NPX rats between 10 and 25 days postnephrectomy to allow for the completion of the rapid phase of compensatory renal growth.

Aliquots of cells were either frozen immediately or were used for enzyme assays (see below for specific protocols). Frozen samples were thawed only once before assays were performed to minimize damage due to repeated freezing and thawing.

Assays

Protein and DNA. Cellular content of protein was determined by the Lowry method (31) using bovine serum albumin (0.1 to 0.6 mg/ml) as a standard. For analysis, aliquots of cells (generally 0.2 ml) were centrifuged for 2 min in a microcentrifuge, supernatants were discarded, and the cell pellets were solubilized by addition of the appropriate amount (1.0 to 2.0 ml) of 0.1 M NaOH. Cellular content of DNA was determined by the fluorescence assay of Sorger and Germinario (32) by measurement of the relative fluorescence of the DNA–diamidinophenylindole complex with 360-nm excitation and 450-nm emission. Samples or standards (calf thymus double-stranded DNA, 0.25 to 2.5 μg/ml) were solubilized by mixing with 2% (v/v) Triton X-100 in 50 mM NaOH. Standards also contained 0.2 mg bovine serum albumin/ml.

GSH-dependent enzymes. γ-Glutamylcysteine synthetase activity was measured with L-α-aminobutyrate as an analogue of L-cysteine. The assay was performed by coupling formation of L-γ-glutamyl-L-α-aminobutyrate and ADP to pyruvate kinase (EC 2.7.1.40), LDH, and ultimately, NADH oxidation and the consequent decrease in absorbance at 340 nm (33). GSSG reductase (EC 1.6.4.2) activity was measured as NADPH oxidation and was detected by the decrease in absorbance at 340 nm (34). GSH peroxidase (EC 1.11.1.9) activity was measured with 0.25 mM H_2O_2 as substrate and was equated to NADPH oxidation, with detection by the decrease in absorbance at 340 nm (35). γ-Glutamyltransferase (EC 2.3.2.2) activity was measured with γ-glutamyl-p-nitroanilide and glycylglycine as substrates, with p-nitroanilide formation being detected at 410 nm (36). γ-Glutamylcysteine synthetase activity was measured either the same day or within 1 day of cell isolation. GSSG reductase and GSH peroxidase activities were measured within 3 days of cell isolation. γ-Glutamyltransferase activity is stable at 0–4°C for prolonged periods of time, but was generally measured within 1 week of cell isolation.

Enzymes related to cellular energetics. Mg^{2+}-dependent and (Na^+ + K^+)-stimulated ATPase activities (EC 3.6.1.4 and 3.6.1.3, respectively) were measured by coupling ATP hydrolysis to pyruvate kinase and LDH and determination of NADH oxidation by the decrease in absorbance at 340 nm (37). Activity inhibited by 0.1 mM ouabain was taken as (Na^+ + K^+)-stimulated ATPase and activity in the presence of Na^+ ions and not inhibitable by ouabain was taken as Mg^{2+}-dependent ATPase. This concentration of ouabain completely inhibits the (Na^+ + K^+)-stimulated ATPase activity (37). Succinate:cytochrome c oxidoreductase (EC 1.3.99.1) activity was measured by coupling succinate oxidation to ferricytochrome c reduction, and activity was quantitated by determining the increase in absorption at 550.5 nm (38). LDH activity was measured with pyruvate and NADH and was quantitated by measuring the decrease in absorbance at 340 nm (39). Alkaline phosphatase (EC 3.1.3.1) activity was determined with p-nitrophenylphosphate as substrate and was quantitated as p-nitrophenol formation by measuring the increase in absorbance at 410 nm (40). The ATPases, succinate:cytochrome c oxidoreductase, and LDH activities were measured within 2 days of cell isolation. Alkaline phosphatase activity is stable at 0–4°C for prolonged periods of time, but was generally measured within 1 week of cell isolation.

Data Analysis

The primary objective of this study was to compare activities of γ-glutamylcysteine synthetase, other critical GSH-dependent enzymes, and selected key enzymes involved in cellular energetics in renal PT and DT cells from SHAM and NPX rats. Data for SHAM and NPX PT cells or DT cells were first analyzed for normality using the Smirnoff–Kolmogorov test. When data for a parameter for corresponding SHAM and NPX animals were found to fit a normal distribution, they were analyzed by the unpaired t test for two independent samples. Data that did not fit a normal distribution were analyzed with the (nonparametric) Mann–Whitney U rank sum test. The level for p was chosen a priori to be less than 0.05.

The content of cellular protein and DNA was normalized to cell number (i.e., mg protein/10^6 cells and μg DNA/10^6 cells, respectively) and data for enzyme activities were normalized to both cell number (i.e., mU/10^6 cells) and to protein content (i.e., mU/mg protein). Although both ways of data expression provide information for the enzyme activities, normalization to cell number is particularly useful because the cell is the functional unit that undergoes the hypertrophic response.

RESULTS

Cellular Protein and DNA Contents

One of the markers indicating that compensatory renal growth has occurred after uninephrectomy and that this growth is predominantly due to cellular hypertrophy rather than hyperplasia is an increase in the cellular content of protein without a change in the intracellular content of DNA. As demonstrated in a previous study (25), we confirmed in the current study that the cellular content of protein was significantly increased (by 54%) in freshly isolated renal PT cells from NPX rats as compared with that in PT cells from SHAM rats (Fig. 1). Additionally, the content of protein was significantly (by 64%) greater in DT cells from NPX rats than in DT cells from SHAM rats, demonstrating that compensatory hypertrophy also occurs in the distal regions of the rat nephron.

In contrast to the increase in cellular content of protein, only modest (i.e., <15%) changes that were not significant occurred in intracellular content of DNA in either PT cells or DT cells after unilateral nephrectomy and compensatory renal growth (Fig. 2), confirming that hyperplasia is not a significant mechanism of compensatory renal growth.

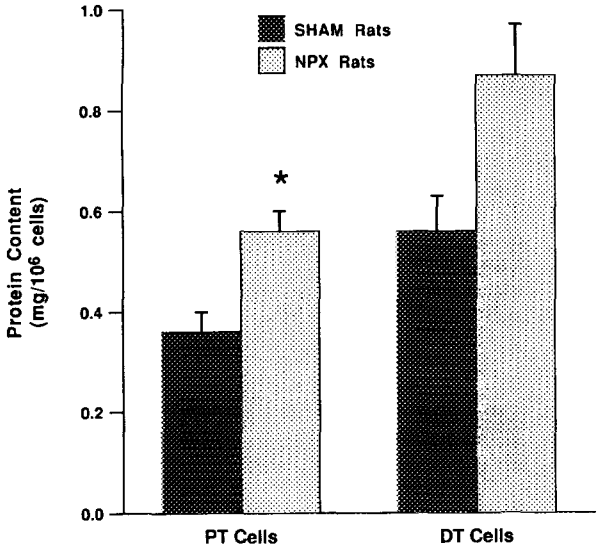

FIG. 1. Effect of uninephrectomy on cellular content of protein in renal PT and DT cells. Cells were isolated from NPX rats between 10 and 25 days postnephrectomy to allow for the completion of the rapid phase of compensatory renal growth. Cellular content of protein was measured by a colorimetric assay in PT and DT cells obtained from SHAM or NPX rats. Results are means ± SE of samples from six SHAM rats or seven NPX rats. *Statistically significant ($P < 0.05$) difference between the mean for the NPX group and the corresponding mean for the SHAM group. For PT/SHAM vs PT/NPX, $P = 0.003$. For DT/SHAM vs DT/NPX, $P = 0.027$.

Activities of GSH-Dependent Enzymes

Our previous finding (22) that the renal concentration of GSH increased significantly as a consequence of uninephrectomy and compensatory renal growth, particularly in the outer stripe of the outer medulla where the proximal straight tubules are localized, led us to determine the effects of compensatory renal hypertrophy on activity of γ-glutamylcysteine synthetase (Fig. 3). The specific activity of γ-glutamylcysteine synthetase per cell was 2.2-fold higher in PT cells isolated from NPX rats than in PT cells from SHAM rats (Fig. 3A). However, the activity of γ-glutamylcysteine synthetase in DT cells from NPX rats was not significantly different from that in DT cells from SHAM rats, indicating that the most prominent effects on GSH metabolism may be restricted to the PT region of the nephron.

As discussed under Experimental Procedures (see Data Analysis), it is useful to express enzyme activities on the basis of both cell number and cellular protein. An increase in activity normalized to cell number indicates a change in enzyme activity per functional unit (i.e., per cell). An increase in activity normalized to cellular protein, however, suggests a prominent role for the enzyme in cellular function. As shown in Fig. 3B, γ-glutamylcysteine synthetase activity in PT cells was significantly increased after compensatory hypertrophy when activity was normalized to cellular content of protein. This increase of approximately 40% suggests a particular importance for this enzymatic activity in the hypertrophied cell.

In addition to examining the activity of GSH biosynthesis, activities of other GSH-dependent enzymes were determined (Table 1). Specific activity per cell of GSSG reductase was also significantly greater (by 94%) in PT cells from NPX rats than that in PT cells from SHAM rats. In DT cells, however, GSSG reductase activity was not increased in cells from NPX rats as compared with that in cells from SHAM rats, again showing a more prominent effect of compensatory hypertrophy on GSH metabolism in PT cells than that in DT cells. GSH peroxidase, like GSSG reductase, is important in maintaining cellular redox status, especially during oxidative stress. Unlike GSSG reductase, however, the specific activity of GSH peroxidase in either PT or DT cells from NPX rats was not significantly different from that in corresponding PT or DT cells from SHAM rats. γ-Glutamyltransferase activity, which is found at severalfold higher levels in PT cells than in DT cells (26, 29) and is critical to renal cellular turnover of GSH, did not change significantly in either cell population as a consequence of compensatory hypertrophy.

None of the activities of these three other GSH-dependent enzymes determined in this study increased significantly in either PT or DT cells from NPX rats as compared with that in PT or DT cells from SHAM rats when activity was normalized to cellular content of protein (Table 2).

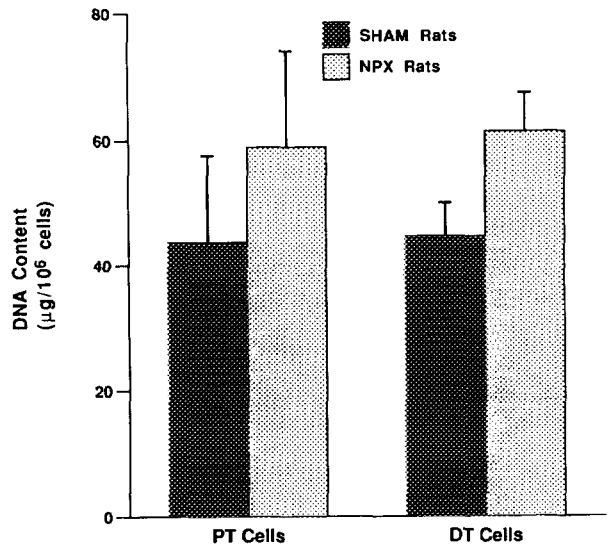

FIG. 2. Effect of uninephrectomy on intracellular content of DNA in renal PT and DT cells. Cells were isolated from NPX rats between 10 and 25 days postnephrectomy to allow for the completion of the rapid phase of compensatory renal growth. Intracellular content of DNA was measured by a fluorimetric assay in PT and DT cells obtained from SHAM or NPX rats. Results are means ± SE of samples from six SHAM rats or seven NPX rats. For PT/SHAM vs PT/NPX, $P = 0.477$. For DT/SHAM vs DT/NPX, $P = 0.066$.

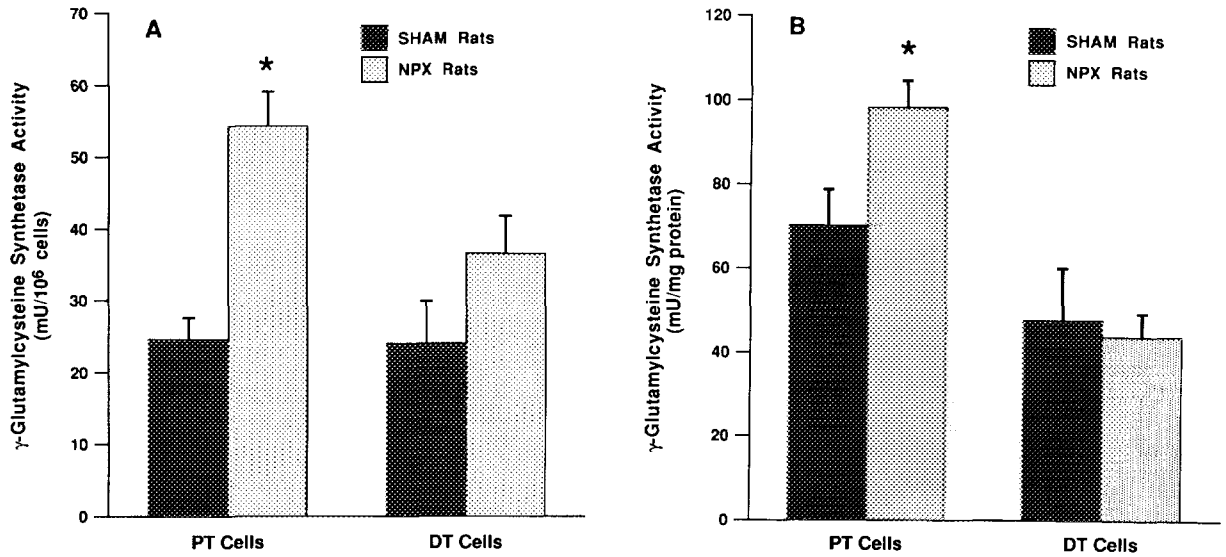

FIG. 3. Effect of uninephrectomy and compensatory renal growth on γ-glutamylcysteine synthetase activity in renal PT and DT cells. γ-Glutamylcysteine synthetase activity was measured by a pyridine nucleotide-linked, spectrophotometric assay in PT and DT cells obtained from SHAM or NPX rats. Results are means ± SE of samples from six SHAM rats or seven NPX rats, and are normalized to cell number (A) or to cellular protein content (B). *Statistically significant ($P < 0.05$) difference between the mean for the NPX group and the corresponding mean for the SHAM group. (A) For PT/SHAM vs PT/NPX, $P = 0.0004$. For DT/SHAM vs DT/NPX, $P = 0.136$. (B) For PT/SHAM vs PT/NPX, $P = 0.020$. For DT/SHAM vs DT/NPX, $P = 0.766$.

Cellular Energy Metabolism

Besides GSH metabolism, energetics is another aspect of renal cellular function that may exhibit prominent changes due to compensatory renal hypertrophy. The $(Na^+ + K^+)$-stimulated ATPase is critical for maintenance of transmembrane Na^+ ion and K^+ ion gradients and for providing the energetic driving force for numerous secondary active metabolite transport systems. Specific activity normalized to cell number was significantly greater (by 120%) in PT cells from NPX rats than in PT cells from SHAM rats (Fig. 4A). This suggests a prominent role for this activity in the adaptation of PT cell function during compensatory hypertrophy. Specific activity of the enzyme was not statistically different between DT cells from NPX rats and DT cells from SHAM rats. Normalization of $(Na^+ + K^+)$-stimulated ATPase activity of cellular protein content showed no significant differences between cells from SHAM and NPX rats, although the P value for the comparison of $(Na^+ + K^+)$-stimulated ATPase activity in PT cells from SHAM rats and that from PT cells from NPX rats was close to statistical significance (i.e., $P = 0.075$; Fig. 4B).

Mg^{2+}-dependent ATPase specific activity per cell was significantly greater in both PT and DT cells derived from NPX rats than in corresponding PT and DT cells derived from SHAM rats (Table 3). The increase in both cell populations from the NPX rats was approximately 70%. Succinate:cytochrome c oxidoreductase activity, which measures the capability of electron transfer over the majority of the electron transport chain, was nearly doubled when normalized to cell number in PT cells from NPX rats as compared with that in PT cells from SHAM rats. Activity in DT cells from NPX rats was not significantly different from that in DT cells from SHAM rats. Increased LDH activity normalized to cell number is a characteristic part of the hypertrophic response in the PT region of the nephron and was confirmed in this study in PT cells (79% increase in specific activity). Alkaline phosphatase, like γ-glutamyltransferase, is a brush-border membrane marker in PT cells. Unlike γ-glutamyltransferase, however (cf. Table 1), alkaline phosphatase activity normalized to cell number was higher in PT cells from NPX rats than in PT cells from SHAM rats, although this difference was not quite significant ($P = 0.06$). There was no significant difference in alkaline phosphatase activity between DT cells from either NPX or SHAM rats.

There were no significant differences in the activities, normalized to cellular content of protein, of any of the enzymes involved in cellular energy metabolism in either PT or DT cells of NPX rats as compared with those in corresponding PT or DT cells from SHAM rats (Table 4).

DISCUSSION

The goals of the current studies were, first, to investigate the effects of compensatory cellular hypertrophy due to uninephrectomy on renal GSH metabolism and, second and more generally, to investigate other biochemical effects of compensatory cellular hypertrophy, not only in PT cells, but in cells of other segments of the nephron

TABLE I

Effects of Compensatory Renal Growth on Activities of GSH-Dependent Enzymes Normalized to Cell Number in Renal PT and DT Cells[a]

	PT cells			DT cells		
	Mean ± SE	*n*	*P* value	Mean ± SE	*n*	*P* value
GSSG reductase (mU/10^6 cells)						
SHAM	6.35 ± 1.72	6		5.95 ± 1.43	6	
NPX	12.3 ± 2.2	7	0.033	9.34 ± 2.22	7	0.244
GSH peroxidase (mU/10^6 cells)						
SHAM	10.1 ± 1.4	6		11.1 ± 1.7	6	
NPX	14.7 ± 3.2	7	0.247	18.1 ± 4.1	7	0.162
γ-Glutamyltransferase (mU/10^6 cells)						
SHAM	97.8 ± 13.7	6		22.6 ± 3.8	6	
NPX	112 ± 22	7	0.6149	26.5 ± 3.7	7	0.470

[a] GSSG reductase and GSH peroxidase activities were measured by pyridine nucleotide-linked spectrophotometric assays. γ-Glutamyltransferase activity was measured with γ-glutamyl-*p*-nitroanilide and glycylglycine as substrates by a spectrophotometric assay. Statistical significance of differences between means in PT or DT cells from SHAM and NPX rats were determined by unpaired *t* tests.

that may also undergo physiological and biochemical changes during compensatory renal growth. In previous work (22, 24), we demonstrated that uninephrectomy and compensatory renal growth cause increases in renal concentrations of GSH, particularly in the outer stripe of the outer medulla, which is where the pars recta of the proximal tubule is localized. Since GSH synthesis by the rate-limiting enzyme γ-glutamylcysteine synthetase is regulated by feedback inhibition, the only way to increase significantly the intracellular concentrations of GSH (besides direct addition of GSH or addition of certain precursors) is by production of new γ-glutamylcysteine synthetase. The hypothesis that we tested in these studies, therefore, is that compensatory renal cellular hypertrophy is accompanied by an induction of the synthesis of γ-glutamylcysteine synthetase.

TABLE II

Effects of Compensatory Renal Growth on Activities of GSH-Dependent Enzymes Normalized to Cellular Protein in Renal PT and DT Cells[a]

	PT cells			DT cells		
	Mean ± SE	*n*	*P* value	Mean ± SE	*n*	*P* value
GSSG reductase (mU/mg protein)						
SHAM	16.9 ± 3.1	6		10.2 ± 1.5	6	
NPX	22.3 ± 3.7	7	0.294	10.4 ± 2.2	7	0.941
GSH peroxidase (mU/mg protein)						
SHAM	28.1 ± 3.0	6		20.7 ± 3.1	6	
NPX	26.8 ± 6.3	7	0.861	20.6 ± 4.0	7	0.992
γ-Glutamyltransferase (mU/mg protein)						
SHAM	276 ± 29	6		41.5 ± 6.0	6	
NPX	199 ± 34	7	0.119	31.9 ± 10.7	7	0.212

[a] GSSG reductase and GSH peroxidase activities were measured by pyridine nucleotide-linked spectrophotometric assays. γ-Glutamyltransferase activity was measured with γ-glutamyl-*p*-nitroanilide and glycylglycine as substrates by a spectrophotometric assay. Statistical significance of differences between means in PT or DT cells from SHAM and NPX rats were determined by unpaired *t* tests.

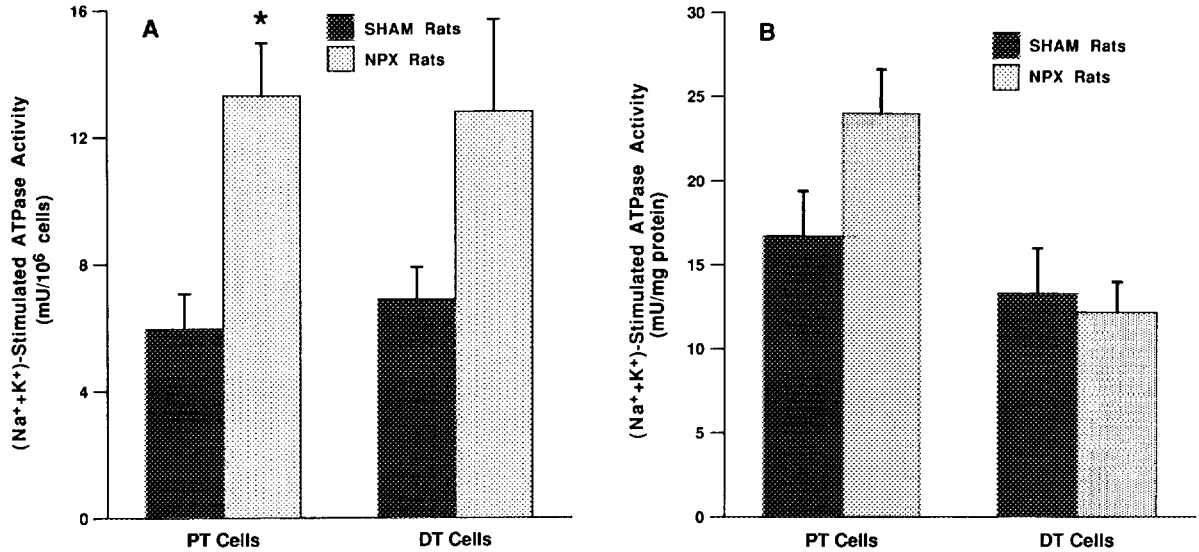

FIG. 4. Effect of uninephrectomy and compensatory renal growth on (Na^+ + K^+)-stimulated ATPase activity in renal PT and DT cells. (Na^+ + K^+)-stimulated ATPase activity was measured by a pyridine nucleotide-linked, spectrophotometric assay in PT and DT cells obtained from SHAM or NPX rats. Activity was calculated as ouabain-sensitive NADH oxidation. Results are means ± SE of samples from six SHAM rats or seven NPX rats and are normalized to cell number (A) or to cellular protein content (B). *Statistically significant ($P < 0.05$) difference between the mean for the NPX group and the corresponding mean for the SHAM group. (A) For PT/SHAM vs PT/NPX, $P = 0.005$. For DT/SHAM vs DT/NPX, $P = 0.102$. (B) For PT/SHAM vs PT/NPX, $P = 0.075$. For DT/SHAM vs DT/NPX, $P = 0.745$.

Measurements of the content of protein and DNA in cells from SHAM and NPX rats demonstrated that compensatory renal cellular hypertrophy occurred, that it was maintained in our *in vitro* model system, and that it was expressed equally in DT and PT cells. The increase in cellular protein content without a corresponding increase in intracellular DNA content indicates that the compensatory response was indeed due predominantly to cellular

TABLE III

Effects of Compensatory Renal Growth on Enzyme Activities Involved in Cellular Energy Metabolism Normalized to Cell Number in Renal PT and DT Cells[a]

	PT cells			DT cells		
	Mean ± SE	*n*	*P* value	Mean ± SE	*n*	*P* value
Mg^{2+}-dependent ATPase ($mU/10^6$ cells)						
SHAM	30.1 ± 7.3	6		35.5 ± 4.9	6	
NPX	50.3 ± 5.3	7	0.044	60.6 ± 9.7	7	0.050
Succinate:cytochrome c oxidoreductase ($mU/10^6$ cells)						
SHAM	22.1 ± 5.7	6		17.9 ± 5.0	6	
NPX	42.2 ± 5.9	7	0.034	27.1 ± 5.9	6	0.266
Lactate dehydrogenase ($mU/10^6$ cells)						
SHAM	57.1 ± 13.5	6		60.4 ± 8.7	6	
NPX	102 ± 16	6	0.039	78.3 ± 16.9	7	0.392
Alkaline phosphatase ($mU/10^6$ cells)						
SHAM	11.7 ± 1.1	6		7.22 ± 1.49	5	
NPX	30.2 ± 7.2	7	0.060	8.41 ± 1.33	7	0.561

[a] ATPase and lactate dehydrogenase activities were determined by pyridine nucleotide-linked spectrophotometric assays. Mg^{2+}-dependent ATPase activity was the ouabain-insensitive ATPase activity. Succinate:cytochrome c oxidoreductase activity was measured spectrophotometrically as cytochrome c reduction and alkaline phosphatase activity was measured spectrophotometrically as *p*-nitrophenol formation. Statistical significance of differences between means in PT or DT cells from SHAM and NPX rats were determined by unpaired *t* tests.

TABLE IV

Effects of Compensatory Renal Growth on Enzyme Activities Involved in Cellular Energy Metabolism Normalized to Cellular Protein in Renal PT and DT Cells[a]

	PT cells			DT cells		
	Mean ± SE	n	P value	Mean ± SE	n	P value
Mg^{2+}-dependent ATPase (mU/mg protein)						
SHAM	81.2 ± 13.5	6		66.9 ± 9.7	6	
NPX	89.8 ± 12.4	7	0.645	70.2 ± 9.6	7	0.811
Succinate:cytochrome c oxidoreductase (mU/mg protein)						
SHAM	61.7 ± 13.8	6		37.5 ± 15.3	6	
NPX	75.4 ± 9.7	7	0.424	24.8 ± 2.8	7	0.434
Lactate dehydrogenase (mU/mg protein)						
SHAM	155 ± 29	6		115 ± 19	6	
NPX	158 ± 15	6	0.944	89.7 ± 16.6	7	0.344
Alkaline phosphatase (mU/mg protein)						
SHAM	43.9 ± 8.5	6		12.4 ± 2.3	5	
NPX	53.7 ± 12.1	7	0.533	9.9 ± 1.2	7	0.314

[a] ATPase and lactate dehydrogenase activities were determined by pyridine nucleotide-linked spectrophotometric assays. Mg^{2+}-dependent ATPase activity was the ouabain-insensitive ATPase activity. Succinate:cytochrome c oxidoreductase activity was measured spectrophotometrically as cytochrome c reduction and alkaline phosphatase activity was measured spectrophotometrically as p-nitrophenol formation. Statistical significance of differences between means in PT or DT cells from SHAM and NPX rats were determined by unpaired t tests.

hypertrophy rather than cellular hyperplasia (1, 2, 9). The reported small degree of hyperplasia that occurs in compensatory renal growth (1) is consistent with the modest, but statistically insignificant increase in both PT and DT cell content of DNA from NPX rats as compared with that from SHAM rats reported here (cf. Fig. 2). It is likely that some of the response to uninephrectomy will be cell division because as cells undergo hypertrophy, there is a tendency to divide when their size reaches a certain point. There is also a variable degree of hypertrophy among the various cells that comprise the PT cell preparation (25). Hence, it is likely that some modest increase in DNA content would be observed in a subfraction of cells. The results overall, however, are consistent with those reported in the literature, which show that hypertrophy is the predominant response observed in renal tubular cells.

Activity of γ-glutamylcysteine synthetase was significantly increased in PT cells that underwent hypertrophy (Fig. 3). The increase was observed both when activity was normalized to cell number and when it was normalized to cell protein. This is important for two reasons: First, the increase per cell shows that the rate of GSH synthesis increases as the size of the cell increases, thereby allowing the cells to at least maintain the same, if not higher, concentrations of GSH; second, the increase per milligram protein indicates that GSH status is particularly important to renal PT cells that have undergone hypertrophy. This correlates with the increased work load per cell due to the enlarged size and higher rate of metabolism (15–17). The absence of a statistically significant increase in γ-glutamylcysteine synthetase activity in DT cells from NPX rats as compared with that in DT cells from SHAM rats, despite other biochemical evidence of hypertrophy, suggests that either DT cells do not need to increase their GSH concentrations or that their GSH metabolic machinery does not have the capacity to respond to the hypertrophy. This last explanation is similar to what we concluded when comparing the response of renal PT and DT cells to oxidative stress (30), namely that PT cells have an inherently greater capacity than DT cells to utilize GSH for drug metabolism reactions and cellular defense mechanisms.

Activities of other GSH-dependent enzymes were also determined. GSSG reductase activity nearly doubled in PT cells after compensatory hypertrophy, but the activity was not changed significantly in DT cells. Neither γ-glutamyltransferase nor GSH peroxidase exhibited any changes in activity after hypertrophy, suggesting that their function is adequate to meet the needs of the hypertrophied PT and DT cells.

Activities of selected enzymes that are critical to cellular energetics were also determined to assess the metabolic state of the hypertrophied cell. Mg^{2+}-dependent ATPase specific activity per cell increased significantly as a consequence of hypertrophy in both PT and DT cells, while specific activity of the $(Na^+ + K^+)$-stimulated ATPase, which is critical for maintenance of cellular ion gradients across plasma membranes, was only increased in PT cells.

Consistent with the increased work load and hence, increased energy requirements that this effect imposes, specific activities of both succinate:cytochrome c oxidoreductase and LDH were increased significantly in PT cells due to hypertrophy. No significant increases occurred in DT cells, again showing that the biochemical changes due to compensatory hypertrophy are more prominent in the PT region than in the DT region. Although the DT cell preparation is 88% free of PT cell contamination (26), it is possible that some of the higher activity values observed in the DT cells may be due to the minimal contamination of these cells with PT cells. Many of the increases in enzymatic activities in DT cells from NPX rats as compared with DT cells from SHAM rats were not statistically significant, indicating that any cross-contamination with PT cells did not affect the results to the point where interpretations or conclusions became ambiguous.

In previous work (22), we showed that both compensatory hypertrophy and subtoxic doses of mercuric chloride increased the intracellular content of GSH, particularly in the outer stripe of the outer medulla. While the present study shows that the increase in intracellular content of GSH after compensatory renal hypertrophy is associated with, and may be due to, an increase in cellular activity of the rate-limiting enzyme of GSH biosynthesis, we have not directly investigated whether the similar increase in intracellular content of GSH after administration of a subtoxic dose of mercuric chloride is also associated with increased activity of GSH biosynthesis. However, we have preliminary data (27 and L. H. Lash and R. K. Zalups, unpublished data) suggesting that administration of a subtoxic dose of mercuric chloride (0.5 μmol/kg) to rats also produces a significant increase in cellular activity of GSH biosynthesis. In support of these preliminary results, Woods *et al.* (41) recently reported that prolonged treatment of male Fischer 344 rats with low doses (up to 10 ppm over 4 weeks) of methyl mercury resulted in a two- to threefold increase in renal GSH concentration and a 4.4-fold increase in mRNA for γ-glutamylcysteine synthetase. They suggested that the increase in GSH synthesis may be an adaptive response to inorganic mercury-induced oxidative stress in the kidneys. Similarly, the increases in activity of GSH biosynthesis reported here after compensatory hypertrophy may be an adaptive response to the hypermetabolic state of renal PT cells. Additional studies, along both the lines of the present report and those that determine directly the effects of Hg on transcription of γ-glutamylcysteine synthetase mRNA and translation of the protein, will be required to demonstrate unequivocally this hypothesis.

Although we showed previously that compensatory renal hypertrophy is associated with increased intracellular content of GSH *in vivo* (22, 24), and this observation was the basis for the hypothesis tested, we did not measure the intracellular content of GSH in the freshly isolated PT and DT cells in the present study. While the collagenase perfusion and Percoll density-gradient centrifugation procedure for isolation of renal PT and DT cells from rats does not appear to alter enzymatic activities as compared with those in the *in vivo* state (25, 26, 28–30), intracellular GSH is rather labile with a half-life of 30 min (28), so that content of GSH in freshly isolated cells may not accurately reflect *in vivo* renal content of GSH. Isolated renal cells are normally preincubated for 15 min at 37°C before additional incubations or assays are performed to allow for the reestablishment of stable conditions (26). Therefore, measurement of intracellular GSH content in the freshly isolated cells may not provide pertinent information about *in vivo* conditions, although it could provide information about direct effects of exogenous chemicals on intracellular GSH status when treatments are performed *in vitro* (30). In a recent study (25), we found only a small increase in GSH content in PT cells from NPX rats as compared with that in PT cells from SHAM rats. The *in vitro* model of compensatory renal hypertrophy was validated because cellular content of protein increased per cell as a consequence of compensatory hypertrophy and other enzymatic activities known to increase, such as LDH, were higher in PT cells from NPX rats as compared with those in PT cells from SHAM rats.

In conclusion, we have demonstrated that PT cells, but not DT cells, isolated from the remnant kidney of NPX rats exhibit marked, significant increases in activities of γ-glutamylcysteine synthetase relative to that in PT cells isolated from the kidneys of control rats. This supports our hypothesis that GSH synthesis is induced during cellular hypertrophy in proximal tubules. Other prominent changes in expression of enzymes involved in drug and intermediary metabolism occur and are more pronounced in PT cells than in DT cells, suggesting that PT cells can more easily adapt their function to the hypertrophic state than DT cells. Future studies on the transcriptional and translational regulation of GSH biosynthesis are warranted to identify the molecular signal responsible for initiating the increase in activity levels of γ-glutamylcysteine synthetase in response to stresses such as compensatory renal hypertrophy.

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