INCREASE IN UREA IN CONJUNCTION WITH L-ARGININE METABOLISM IN THE LIVER LEADS TO INDUCTION OF CYTOCHROME P450 2E1 (CYP2E1): THE ROLE OF UREA IN CYP2E1 INDUCTION BY ACUTE RENAL FAILURE

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ABSTRACT:

A number of xenobiotics and certain pathophysiological situations cause the induction of CYP2E1. The present study was designed to establish the role of plasma urea nitrogen and l-arginine on hepatic CYP2E1 expression in rats or rats with acute renal failure. Exposure of rats to a single intravenous dose of 5 mg/kg uranyl nitrate caused renal failure in 5 days (ARF), as evidenced by increases in plasma urea nitrogen level and kidney to body weight ratio. Northern and Western blot analyses revealed that hepatic CYP2E1 was 2- to 4-fold induced by ARF. Treatment of rats with either 10% glucose in drinking water for 5 days following a single injection of uranyl nitrate or two injections of recombinant growth hormone (5 units/kg, s.c., twice a day) on the 4th day after uranyl nitrate injection reduced both the rise in plasma urea nitrogen and the induction of CYP2E1. Exposure of rats to urea (~225 mg/kg/day) in drinking water for 1 to 3 day(s) resulted in significant increases in CYP2E1 mRNA and protein. Furthermore, perfusion of the liver with 25 mM urea for 24 h resulted in CYP2E1 induction with an increase in the mRNA. The levels of CYP2E1 protein and mRNA were increased in rats perfused with 25 mM l-arginine for 24 h (i.e., a 4-fold increase). Hence, l-arginine, which is irreversibly hydrolyzed to urea and ornithine by arginase, also induced hepatic CYP2E1. The results of the present study provided evidence that increases in plasma urea in conjunction with l-arginine metabolism lead to the induction of CYP2E1 in the liver.

Cytochrome P450 2E1, CYP2E1, is an ethanol-inducible form of cytochrome P450 with broad substrate specificity and is inducible by a number of small organic molecules including therapeutic agents and toxicants (Hong et al., 1987; Kim and Novak, 1993; Lieber, 1997). Certain pathophysiological situations such as diabetes mellitus, hypophysectomy, and starvation with coprophagy also result in CYP2E1 induction (Chung et al., 2001b). The metabolic activity of CYP2E1 is closely related with production of reactive oxygen as a result of the high rate of uncoupling of electron transfer and oxygen reduction by CYP2E1 (Bell and Guengerich, 1997; Lieber, 1997). CYP2E1 is predominantly in the form of high-spin state and thus generates reactive oxygen species (Bell and Guengerich, 1997; Lieber, 1997). Hence, CYP2E1 induction increases production of reactive oxygen species and stimulates lipid peroxidation (Wu and Cederbaum, 2001). A recent study showed that hepatic stellate cells cocultured with HepG2 cells expressing CYP2E1 are activated for the production of collagen to a greater extent than control cells (Nieto et al., 2002), raising the possibility that CYP2E1 induction is associated with liver fibrogenesis (i.e., synthesis of collagen). The mechanisms governing regulation of CYP2E1 expression are complex and involve transcriptional, post-transcriptional, translational, and post-translational events (Kim et al., 1990; Woodcroft and Novak, 1997). Expression of CYP2E1 in hepatocytes is controlled by hormones including insulin, thyroid hormone, and growth hormone (Waxman et al., 1989; Peng and Coon, 1998; Son et al., 2000). Glucose utilization seemed to be an important determinant for CYP2E1 expression. We previously reported that CYP2E1 expression is highly dependent on the plasma glucose level (Son et al., 2000) in association with the aforementioned hormones. We also showed that starvation in combination with coprophagy resulted in the increases in CYP2E1 mRNA and protein (Chung et al., 2001b). CYP2E1 expression is also affected by the change in energy metabolism. γ-Ray irradiation at the exposure level inducing organelle dysfunction induced CYP2E1 in the liver, which paralleled with mitochondrial damage but not with alterations in glucose or insulin levels (Chung et al., 2001a). CYP2E1 is inducible in the kidney as well. The regulatory mechanism governing CYP2E1 expression in the kidney appeared to differ from that in the liver. For instance, pyridine induces CYP2E1 without an increase in the mRNA, whereas renal CYP2E1 is inducible by pyridine with the mRNA elevation (Kim et al., 1992). This discrepancy may result from the tissue-specific production of endogenous metabolites, which may affect the expression of CYP2E1. In spite of the extensive studies on the regulation of CYP2E1 expression, the molecular mechanistic basis for the enzyme induction has not been completely delineated yet.

The liver plays a central role in the maintenance of metabolic homeostasis and is the major site of amino acid interconversion. Oxidative deamination results in the conversion of amino acids to
ammonia and α-keto acids. Waste nitrogenous products (i.e., urea and ammonia) exclusively produced in the liver are excreted via the kidney. Urea production is intimately related with the ammonia) exclusively produced in the liver are excreted via the kidney. Urea production is particularly intimately related with the ammonia and urea-perfused, and α-arginine (or α-leucine)-perfused. The portal vein was cannulated by the modified Suzuri method (Xu et al., 1992; Kim et al., 1997). We demonstrated that CYP2E1 was induced in rats with ARF. The role of urea and α-arginine metabolism in the induction of hepatic CYP2E1 was further studied in rats administered with urea and perfused with urea or α-arginine.

Experimental Procedures

Materials. Alkaline phosphatase-conjugated donkey anti-goat IgG was supplied from Invitrogen (Carlsbad, CA). [α-32P]dCTP (3000 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Random prime-labeling kit was obtained from Promega (Madison, WI). Recombinant human growth hormone (rGH) was supplied by the Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea). Uranyl nitrate and most of the reagents in the molecular studies were purchased from Sigma-Aldrich (St. Louis, MO).

Animal Treatment. Five-week old male Sprague-Dawley rats (200 ± 20 g) were supplied from Daehan Laboratory Animals (Eumsung, South Korea). Animals were maintained in a clean room at the Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University (Seoul, South Korea), at a temperature between 20° and 23°C with 12-h light and dark cycles and a relative humidity of 50%. Animals were individually housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and water ad libitum. Rats were randomly divided into five groups: control, ARF, ARF + rGH, ARF + glucose, and urea-treated animal groups. For control group, 0.9% NaCl-injectable solution, 1 ml/kg body weight was injected via the tail vein; for ARF group, uranyl nitrate (dissolved in 0.9% NaCl-injectable solution to make 5 mg/ml), 5 mg/kg body weight, was injected via the tail vein; for ARF + rGH after injection of uranyl nitrate, rGH (diluted with 0.9% NaCl-injectable solution), 5 IU/kg, was injected subcutaneously twice a day on the 4th day after uranyl nitrate for ARF + glucose group, with the injection of uranyl nitrate, glucose (dissolved in drinking water to make 10% w/v) was supplied in drinking water for 5 days; and for urea-treated group, urea (dissolved in drinking water to make 2 g/l) was supplied in drinking water for 1 to 3 day(s). Food and water intakes, body weight, urine output, and amount of feces were measured everyday during experiments. Experiments were performed on the 5th day after an intravenous injection of uranyl nitrate or 0.9% NaCl-injectable solution; liver was exercised for measurement of CYP2E1 expression; and heparinized blood was collected directly by heart puncture for plasma chemistry data, such as urea nitrogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, and insulin. Additional rats were randomly divided into groups; control, urea-perfused, and α-arginine (or α-leucine)-perfused. The portal vein was cannulated by the modified Suzuri method (Xu et al., 1992; Kim et al., 1997). After midline abdominal incision, the middle portion of the portal vein was isolated, and the tapered end of a 23-gauge needle, bent at 60° angle, was inserted into the pyloric vein (to minimize the impairment of blood flow in the portal vein). Bleeding was prevented by applying epoxy glue (Krazy Glue; Krazy Glue Inc., Itasca, IL). A 5-cm piece of the silastic tubing (Dow Corning, Midland, IL) was attached to the other end of the needle, which linked with the dorsal side cannula of the neck. The silastic tubing was covered with a wire to allow free movement of the rat. The exposed area was closed using surgical suture. Each animal was housed individually in a metabolic cage for 2 to 3 h to recover from anesthesia. Animals were not restrained at any time during the study. Heparinized 0.9% NaCl-injectable solution (20 units/ml, 0.3 ml) was used to flush each cannula to prevent blood clotting. Rats were infused with 25 mM urea or 25 mM α-arginine (or α-leucine) as dissolved in 0.9% NaCl for 24 h with the total infusion rate and volume of 0.6 ml/h and 14.4 ml, respectively. Total amounts of urea and α-arginine infused were 114 and 315 mg/kg, respectively. Level of CYP2E1 expression and plasma chemistry were assessed as described previously. Assays were performed with the samples prepared from individual animals. Blood was collected from the heart and analyzed for the contents of glucose, insulin, plasma urea nitrogen, creatinine, and aminotransferase activities. Data points represent mean ± S.D. from four to six animals.

Preparation of Microsomal Proteins. Hepatic microsomal fractions were prepared by differential centrifugations at 10,000 g for 30 min and then at 100,000 g for 90 min. Microsomes were washed in pyrophosphate buffer and stored in 50 mM Tris-acetate buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol, as described previously (Kim et al., 1996). Protein contents were assayed (Lowry et al., 1951), and the subcellular preparations were stored at −70°C until use.

Immunoblot Analysis. Sodium dodecylsulfate (SDS)-polyacylamide gel electrophoresis and immunoblot analyses were performed according to the previously published procedures (Kim et al., 1996). Microsomal proteins were separated by a 7.5% gel and electrophoretically transferred to a nitrocellulose paper. The nitrocellulose paper was incubated with anti-CYP2E1 antibody (Oxford Biomedical Research, Oxford, MI; Kim et al., 1996), followed by incubation with alkaline phosphatase-conjugated secondary antibody and developed using 5-bromo-4-chloro-3-indolylphosphate and 4-nitroblue tetrazolium chloride.

Northern Blot Hybridization. Specific cDNA probes for the CYP2E1 gene were amplified by reverse transcription-polymerase chain reaction using the selective primers (Kim and Novak, 1993), cloned in the pGEM + T vector (Promega), as described previously (Cho et al., 1999). The expression of CYP1A2, CYP2B1/2, and CYP2C11 was also assessed by Northern blot analyses using the specific cDNA probes, as described previously (Chung et al., 2001a; Kim et al., 2001). Total RNA was isolated using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction according to the method of Puissant and Houdebine (1990). Northern blot analysis was carried out according to the procedure, as described previously (Kim and Cho, 1996). Briefly, total RNA isolated from the liver was harvested by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde and transferred to a nitrocellulose paper. The nitrocellulose paper was baked in a vacuum oven at 80°C for 2 h. The blot was hybridized, as described previously (Kim and Cho, 1996). Filters were washed in 2× standard saline citrate and 0.1% SDS for 10 min at 42°C three times and in 0.1× standard saline citrate and 0.1% SDS for 10 min at 42°C twice. Filters were finally washed in the solution containing 0.1× standard saline citrate and 0.1% SDS for 60 min at 55°C. After quantification of mRNA levels, the membranes were stripped and rehybridized with a labeled probe complementary to 18S rRNA to quantify the amount of RNA loaded onto the membranes.

Plasma Chemistry. The plasma level of urea nitrogen, creatinine, glucose, insulin, ALT, and AST were colorimetrically assayed using commercially available kits (Roche Applied Science, Indianapolis, IN) or measured by a 747 Automatic Analyzer (Hitachi, Tokyo, Japan).

p-Nitrophenol (PNP) Hydroxylase Activity. All reactions were conducted under conditions that were linear with respect to time and protein concentration at 37°C. The hydroxylation of PNP was determined spectrally as described by Koop et al. (1997). Reaction mixtures (a final volume of 1 ml) that contained 100 μM PNP, 100 mM potassium phosphate buffer (pH 6.8), and 0.2 mg of microsomal proteins were initiated by the addition of 1 mM NADPH. Reactions were terminated by the addition of 0.2 ml of 1.5 M perchloric acid at 10 min.

HPLC Analysis of Chlorzoxazone and 6-Hydroxychlorzoxazone. The concentrations of chlorzoxazone and 6-hydroxychlorzoxazone were analyzed by the reported HPLC method (Frey and Stiffl, 1996). To 0.05 ml of sample were added 0.1 ml of 0.2 M sodium acetate buffer (pH 4.75) and 200 units of...
β-glucuronidase dissolved in 0.1 ml of isotonic phosphate buffer (pH 7.4). Samples were manually mixed and incubated in a water-bath shaker kept at 37°C and at a rate of 50 oscillation per min for 2 h. After incubation, 0.05 ml methanol containing 10 μg/ml 3-aminophenol sulfone was added, vortex-mixed, and 1 ml of diethyl ether was added. The mixture was shaken for 10 min and centrifuged at 2000 g for 10 min. The upper organic layer was transferred to a clean tube and evaporated at 37°C under a stream of nitrogen. The residues were reconstituted in 100 μl of the mobile phase and a 50-μl aliquot was injected directly onto the HPLC column. The mobile phase, 0.1 M ammonium acetate/acetonitrile/tetrahydrofuran (72:22:5, v/v/v) was run at a flow rate of 1.5 ml/min. A UV detector was set at 283 nm monitored the column effluent. The retention times for 6-hydroxyclorozoxazone, 3-aminophenol sulfone, and chlorozoxazone were approximately 6, 10, and 18 min, respectively. The coefficients of variation of the assay (within- and between-day) were generally low (below 8.2%).

Cytochrome P450 Content. The content of cytochrome P450 in liver microsomes was determined by its carbon monoxide spectrum difference after reduction with sodium dithionite according to the reported method (Omura and Sato, 1964) using a spectrophotometer (UV-260; Shimadzu, Kyoto, Japan). An extinction coefficient of 91 mM/cm was used to calculate the cytochrome P450 content of the liver.

NADPH-Dependent Cytochrome c Reductase Activity and Cytochrome b5 Content. The activity of NADPH-dependent cytochrome c reductase was measured using cytochrome c as a substrate (Wallace, 1994), and cytochrome b5 content was determined from its redox spectrum of NADH-reduced versus NADH-oxidized cytochrome (Snell and Mullock, 1987).

HPLC Assay of L-Arginine in the Liver. L-Arginine was analyzed according to the method of Rajendra (1987) with slight modification. Briefly, the liver was homogenized in 10 volumes of cold methanol, and homogenate was quickly mixed with 3 volumes of cold methanol for determination of l-arginine. The denatured proteins were removed by centrifugation at 10,000 g for 10 min. l-Arginine was derivatized with o-phthalaldehyde/2-mercaptoethanol and analyzed using reversed-phase HPLC (model PU-980 pump; Jasco, Tokyo, Japan, and model FP-920 fluorescence detector; Jasco) equipped with 3.5-μm Kromasil C18 (4.6 × 100 mm; Eka Chemicals, Bohus, Sweden).

Data Analysis. Scanning densitometry of Northern and Western blots was performed with the Image Scan and Analysis System (α-Innotech, San Leandro, CA) to assess the expression of CYP2E1. Data were analyzed using the SigmaStat program (SPSS Inc., San Rafael, CA) and expressed as means ± S.D. For molecular study, one-way analysis of variance procedures were used to assess significant differences (P < 0.05 or 0.01) among treatment groups. For each significant effect of treatment, the Newman-Keuls test was used for comparisons of multiple group means. The plasma chemistry data were analyzed using the Duncan’s multiple range test of SPSS posteriori one-way analysis of variance program among the three to five means for unpaired data. All data were expressed as mean ± S.D.

Results

Induction of ARF. In the ARF rats, the impaired kidney function was obvious; the level of urea nitrogen (10.3 times increase) (Table 1) and kidney weight based on body weight (60% increase) were significantly greater than those in control rats (i.e., kidney weight, 9.17 ± 0.50 and 14.7 ± 1.06 g/kg body weight in control and ARF rats, respectively). Similar results were also reported elsewhere (Kim et al., 1998). The plasma levels of AST (66.9% increase) and insulin (36.4% increase) were significantly higher in ARF rats than those in control rats (Table 1).

CYP2E1 Induction in ARF Rats. Expression of hepatic CYP2E1 was monitored in ARF rats. Northern blot analysis revealed that the CYP2E1 mRNA was 4-fold increased by ARF, as compared with control (Fig. 1). Immunoblot analysis showed that CYP2E1 protein was also >2-fold induced (Fig. 1). The rates of liver microsomal PNP metabolism were 1.02 ± 0.30 and 1.77 ± 0.50 nmol 4-nitrocatechol/min/mg of proteins in control and ARF rats (P < 0.01), respectively. A lesser increase in PNP activity than that in immunoblot could result from the contribution of other inducible cytochrome P450s and the lack of complete substrate specificity (Zerilli et al., 1998). To better correlate changes in mRNA and protein with the catalytic activity of CYP2E1, chlorozoxazone hydroxylase activity, which is known to be more specific than PNP (Peter et al., 1990; Koop, 1992), was measured in the microsomes prepared from rats with ARF (Table 2). The Vm value for the formation of 6-hydroxyclorozoxazone from chlorozoxazone by microsomes prepared from livers of control rats and rats with ARF was 2.59 ± 1.66 and 5.4 ± 2.88 nmol/min/mg of proteins, respectively; although the Km value was comparable (144 ± 110 and 131 ± 65 μM). Intrinsic clearance of chlorozoxazone hydroxylase activity was ~2.2-fold increased in the livers of ARF rats, as compared with control (0.0198 ± 0.0113 and 0.0428 ± 0.0101 ml/min/mg of proteins in control and ARF rats, respectively; P < 0.05). These data raised the possibility that increase in plasma nitrogenous waste products leads to CYP2E1 induction in ARF rats.

We further determined the effects of ARF on the expression of other forms of cytochrome P450. Northern blot analyses revealed that ARF suppressed the expression of CYP2C11 (0.58 ± 0.04 of control), whereas CYP1A2, CYP2B1/2, or CYP3A2 was not significantly affected by ARF. Total cytochrome P450 content, NADPH-dependent
Rats were exposed to a single dose of 5 mg/kg uranyl nitrate to induce ARF. CYP2E1 expression was monitored on the 5th day after the injection of uranyl nitrate. Northern blot analyses were carried out with total RNA fractions (20 μg of each) prepared from the liver to monitor the mRNA and protein levels for CYP2E1. The amount of RNA loaded in each lane was confirmed by rehybridization of the stripped membrane with a 32P-labeled probe complementary to 18S rRNA. Western blot analysis for CYP2E1 was performed with 15 μg of hepatic microsomal proteins per lane. For details, see Experimental Procedures.

![Expression of hepatic CYP2E1 in ARF rats.](image)

**Fig. 1.** Expression of hepatic CYP2E1 in ARF rats.

Cytochrome c reductase activity, and cytochrome b₅ were all significantly increased in rats with ARF (Table 2). In particular, the level of cytochrome b₅ was >3-fold increased by ARF compared with control (Table 2).

**Effect of rGH on CYP2E1 Induction in ARF Rats.** Hypophysectomy induced CYP2E1, and treatment with rGH to hypophysectomized rats prevented CYP2E1 induction (Son et al., 2000). Both Northern and Western blot analyses revealed that the induction of CYP2E1 was reduced by rGH in ARF rats (Fig. 2). The microsomal metabolic activity of PNP was also reduced by rGH to 1.02 ± 0.26 nmol 4-nitrocatechol/min/mg of proteins, as compared with 1.77 ± 0.5 nmol 4-nitrocatechol/min/mg of proteins in ARF rats (P < 0.05). Treatment of ARF rats with rGH also caused a decrease in intrinsic clearance of chloroazobenzene hydroxylase activity (0.0428 ± 0.0101 versus 0.0231 ± 0.0125 ml/min/mg of proteins; P < 0.05). We then determined the effects of rGH on the urea nitrogen increase in ARF rats (Table 1). An increase in plasma urea nitrogen in ARF rats was 31.7% reduced by two injections of rGH (5 IU/kg body weight, s.c., twice a day) (Table 1). The plasma level of creatinine was also significantly lower (18.3% decrease) by rGH (Table 1). Whereas L-arginine content in the liver was smaller in ARF rats (228 ± 49.2 and 199 ± 55.4 nmol/g wet liver in control and ARF rats, respectively), rGH treatment in ARF rats caused an increase in hepatic L-arginine content (256 ± 11.5 nmol/g wet liver; P < 0.05). It is probable that rGH stimulated protein synthesis and increased positive nitrogen balance, which would concomitantly decrease catabolism of L-arginine.

**Effect of Glucose on the CYP2E1 Induction in ARF Rats.** Previous studies have shown that glucose administration to rats prevented the induction of CYP2E1 by starvation or by water deprivation (Son et al., 2000; Kim et al., 2001). Expression of CYP2E1 was monitored in rats with ARF. Increases in CYP2E1 protein and mRNA levels were significantly reduced by glucose supplementation (Fig. 3), which probably resulted from the improvement in blood chemistry and kidney function. We were also interested in assessing whether exposure of rats to glucose in drinking water affected kidney function and plasma chemistry in ARF rats. Although the kidney weight and the plasma AST activity were not significantly reduced (5.4% and 7.1%, respectively), the plasma urea nitrogen level in ARF rats supplemented with glucose was smaller (18% decrease) than that in ARF rats. These data showed that glucose and presumably its utilization affected plasma urea nitrogen in ARF rats (Table 1). The plasma insulin was not significantly changed by glucose in ARF rats.

**Effect of Urea on CYP2E1 Expression.** Because plasma urea nitrogen was markedly increased in ARF rats, the effect of urea on hepatic CYP2E1 expression was examined. Rats exposed to urea in drinking water for 1 to 3 day(s) exhibited 4- to 6-fold increases in the CYP2E1 mRNA (Fig. 4). CYP2E1 protein was also >2-fold induced relative to control. Blood chemistry was monitored following exposure of rats to urea in drinking water (2 g/l) for 3 days (i.e., the average daily amount ingested was ~225 mg/kg body weight). Exposure of rats to urea significantly increased the plasma insulin level (100% increase) but not the plasma glucose, urea nitrogen, and creatinine levels (Table 1). We first expected the rise in plasma urea nitrogen by urea, which, however, was not increased in rats presumably because of the intact kidney function. This would result from the compensatory excretion of urea. The urea level in the portal blood and liver would, however, be persistently greater than that in the systemic blood because urea was orally administered in this experiment.

**Effect of Urea or L-Arginine Perfusion on CYP2E1 Expression.** To further confirm the effect of urea on CYP2E1 induction, rats were perfused with urea for 24 h via the portal vein. In this experiment, rats were infused with urea dissolved in 14.4 ml of 0.9% NaCl-injectable solution and restricted from foods and drinking water to eliminate other factors and to minimize variations. Control rats were sham-operated and infused with the same volume of normal saline. Because the difference in plasma urea nitrogen level between control and ARF rats was ~150 mg/dl (i.e., equivalent to 25 mM of urea) (Table 1), the same amount of urea (25 mM) was perfused for 24 h. Both CYP2E1 mRNA and protein levels were 3- to 4-fold increased in rats perfused with urea (Fig. 5). The relative CYP2E1 mRNA level was 2-fold increased 8 h following urea perfusion (data not shown). The metabolic activity of PNP in the hepatic microsomes prepared from sham-operated control rats and rats perfused with urea were 0.65 ± 0.32 and 1.10 ± 0.38 nmol 4-nitrocatechol/min/mg of proteins (P < 0.05), respectively. The data were consistent with Western blot analyses (Fig. 5) although the increase in PNP activity was smaller than that in CYP2E1 apoprotein. These results provided evidence that an increase of urea, particularly in the liver, was directly responsible for the induction of CYP2E1 and that CYP2E1 induction by urea accompanied the increase in its mRNA (Fig. 5). Rats perfused with urea showed a 2.5-fold increase in the plasma urea nitrogen (Table 3). An additional study showed that rGH treatments (5 IU/kg body weight, s.c., twice a day) partially reversed the induction of CYP2E1 in rats perfused with urea (data not shown).

Because urea is irreversibly produced by arginase from L-arginine via the urea cycle, studies were extended to determine whether L-arginine was capable of changing plasma urea nitrogen and inducing hepatic CYP2E1 (Table 3; Fig. 5). Perfusion of 25 mM L-arginine for 24 h via the portal vein caused increases in the hepatic CYP2E1 mRNA and protein. The extent of CYP2E1 induction by L-arginine was comparable to that by urea (Fig. 5). Perfusion with 25 mM L-leucine, which was used as a control, did not induce CYP2E1 (data not shown). The rates of liver microsomal PNP metabolism were 0.65 ± 0.32 and 0.96 ± 0.44 nmol 4-nitrocatechol/min/mg of proteins for sham-operated control and L-arginine-perfused rats, respectively. L-Arginine perfusion increased plasma urea nitrogen (Table 3), which was inversely related with the L-arginine content in the liver (427 ± 23, 334 ± 16, and 347 ± 33 nmol/g liver in sham-operated control, urea-perfused and L-arginine-perfused rats, respectively; P < 0.05).

We determined whether L-arginine might alter the catalytic activity of
PNP hydroxylase in vitro. Interestingly, the metabolic activity of PNP in pyrazine-induced hepatic microsomes (i.e., CYP2E1-induced) was significantly increased in the presence of L-arginine in a concentration-dependent manner. L-Arginine at the concentrations of 3 to 100 mM increased the production of 4-nitrocatechol from PNP up to 2-fold [i.e., control, 2.51 ± 0.32; 3 mM L-arginine, 2.81 ± 0.29; 10 mM L-arginine, 3.15 ± 0.50; 30 mM L-arginine, 4.11 ± 0.21 (P < 0.01); and 100 mM L-arginine, 4.92 ± 0.33 (P < 0.01) nmol/min/mg of proteins].

Discussion

Previous studies have shown that CYP2E1 was induced by certain pathophysiological situations (Son et al., 2000; Chung et al., 2001a,b), which involved metabolic disturbance, hormonal change, and toxic insult to the liver. In the present research, we studied for the first time whether hepatic CYP2E1 was induced in ARF rats. Both Northern and Western blot analyses revealed that CYP2E1 was distinctly induced by ARF, which accompanied a concomitant increase in its mRNA. Because CYP2E1 catalyzes a number of xenobiotics including therapeutic agents and toxicants and produces reactive metabolic intermediates, the fact that ARF induces CYP2E1 should be taken into consideration as a pathophysiological and pharmacokinetic factor for the individuals with acute or chronic renal failure. Previous studies showed that discrepancy exists in the induction of CYP2E1 by fasting between animals and humans (Smith et al., 1998) and that coprophagy was the determining factor in animals (Chung et al., 2001b). Whether renal failure affects CYP2E1 expression in humans remains to be further confirmed.

Mitochondria are the major cellular sources of reactive oxygen species and contain the circular double-stranded genome replicated within mitochondria. The mitochondrial DNA is particularly vulner-

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ARF*</th>
<th>ARF + rGH†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450 content (nmol/mg protein)</td>
<td>0.862 ± 0.448</td>
<td>1.40 ± 0.143</td>
<td>0.982 ± 0.265††</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase (nmol cytochrome c reduced/min/mg protein)</td>
<td>315 ± 28.0</td>
<td>581 ± 117**</td>
<td>350 ± 176††</td>
</tr>
<tr>
<td>Cytochrome b$_5$ (nmol/mg protein)</td>
<td>0.595 ± 0.122</td>
<td>1.97 ± 0.660**</td>
<td>0.667 ± 0.180††</td>
</tr>
</tbody>
</table>

* Rats were intravenously injected with a single dose of 5 mg/kg uranyl nitrate.

†† Rats were intravenously injected with a single dose of 5 mg/kg uranyl nitrate, and rGH (5 IU/kg in saline, s.c.) was injected twice on the 4th day after treatment with uranyl nitrate.

* P < 0.05, significant as compared to control.

** P < 0.01, significant as compared to control.

†† P < 0.01, significant as compared to ARF.
able to the damaging effects mediated by reactive oxygen species (Yakes and Houten, 1996; Croteau et al., 1997). Previous studies revealed that mitochondrial dysfunction such as reduction in aconitase activity and decrease in mtDNA in rats irradiated with \( \gamma \)-rays accompanied CYP2E1 induction in the liver (Chung et al., 2001a). This raised the contention that CYP2E1 is inducible in hepatocytes with injuries of energy-producing organelles.

Mitochondria are the organelles producing urea through the urea cycle. The urea cycle is the critical biochemical pathway for the metabolism of amino acids. Because mitochondrial arginase in the liver irreversibly catalyzes the catabolism of L-arginine for the production of urea, which is eventually excreted through the kidney, the capability of renal excretion of urea would be affected by the liver and the kidney function. We found that the plasma AST level was increased in ARF rats, which may result from nitrogenous waste products accumulated by ARF and cell injury. In view of the relationship between the liver and the kidney function as well as the induction of hepatic CYP2E1 by ARF, we investigated the role of urea in the induction of hepatic CYP2E1. Because CYP2E1 induction by ARF accompanied a marked increase in the plasma urea level, we first allowed rats to take urea in drinking water for 1 to 3 days. In spite of no change in plasma urea nitrogen by oral exposure of rats to urea, CYP2E1 was induced in the liver. The lack of increase in plasma urea nitrogen would be due to variable gastrointestinal absorption and/or increase in renal excretion of urea in rats with the intact kidneys. In general, the level of plasma urea nitrogen is not increased in animals with intact kidney. Urea ingested in the drinking water is transported to the systemic circulation through the portal vein. The concentration of urea in the portal vein and hence in the liver would be greater than that in the systemic circulation. To minimize variations in gastrointestinal absorption of urea, rats whose portal veins were cannulated were infused with urea for 24 h. The amount of urea perfused was the same as the difference in plasma urea nitrogen between control and ARF rats. The urea perfusion experiment provided strong evidence that urea was indeed the determining factor for the induction of hepatic CYP2E1. Induction of CYP2E1 by urea also involved an increase in its mRNA, which was also consistent with that by ARF. Many xenobiotics such as solvents, toxicants, and other small organic molecules induce CYP2E1. Whether the xenobiotics inducing CYP2E1 also affect mitochondrial function and hence the plasma urea level should be further investigated.

L-Arginine is the precursor of urea in the mitochondria. Given the metabolic pathway, we assessed whether L-arginine was also capable of inducing CYP2E1 in the liver. Perfusion of the liver with L-arginine equivalent to urea concentration caused induction of CYP2E1 with an increase in the mRNA. These results further confirmed that CYP2E1 was inducible as a result of disturbance of the urea cycle. We also measured the effect of urea or L-arginine on the metabolic activity of PNP in pyridine-induced (i.e., CYP2E1-induced) liver microsomes in vitro. The rate of PNP oxidation to 4-nitrocatechol was increased by the presence of L-arginine, as shown in the present study. The fact that CYP2E1-mediated catalytic activity is increased by 25 mM of L-arginine, but not by 25 mM of urea, raised the possibility that CYP2E1 may interact with L-arginine. Whereas the hepatic content of

**Fig. 4.** Hepatic CYP2E1 mRNA and protein levels in rats exposed to urea (2 g/l) in drinking water for 1 to 3 days.

CYP2E1 mRNA and protein levels were measured, as described in Fig. 1. Data represent the mean ± S.D. with four to five separate experiments. Significant as compared with control; ***, \( P < 0.01 \); *, \( P < 0.05 \).

**Fig. 5.** Hepatic CYP2E1 mRNA and protein levels in rats perfused with 25 mM urea or 25 mM L-arginine for 24 h through portal vein.

Control rats were sham-operated. Expression of CYP2E1 was monitored after infusion of urea or L-arginine for 24 h. Total amounts of urea and L-arginine infused were 114 mg/kg and 315 mg/kg, respectively. CYP2E1 mRNA and protein levels were measured, as described in Fig. 1. Data represent the mean ± S.D. with four to five separate experiments. Significant as compared with control; ***, \( P < 0.01 \).
L-arginine was slightly decreased in ARF rats, treatment of ARF rats with rGH, which reduces the catabolism of amino acids including L-arginine, significantly enhanced hepatic L-arginine content (i.e., the amount of L-arginine as constituents of hepatic proteins). Hence, the content of L-arginine in the liver is inversely related with that in plasma urea nitrogen. rGH also inhibited the induction of CYP2E1 by ARF. These data support the conclusion that disturbance of the metabolic pathway of L-arginine in conjunction with the urea cycle may be tightly coupled with the induction of CYP2E1. Alternatively, nitric oxide produced from L-arginine by nitric oxide synthase may be involved in the induction of CYP2E1. Recently, it was reported that NO from inducible nitric oxide synthase increases the expression of CYP2E1 in inducible nitric oxide synthase-null hepatocytes (Zamora et al., 2001). Nonetheless, it is uncertain whether NO, which would be produced by the catalysis of constitutive nitric oxide synthase activity in the present ARF animal model, is associated with the induction of CYP2E1 by ARF.

In the previous study, we compared the effects of the protein content of the diet on representative cytochrome P450s in rats. Two groups were fed either the control diet containing 23% casein or the low protein diet containing 5% casein for 4 weeks (Cho et al., 1999). CYP2E1 expression was suppressed by feeding the low protein content of the diet. This may have resulted from the diminished supply of L-arginine. Treatments of rats with cysteine (250 mg/kg/day, for 1 to 3 days), which was used as a control, caused no significant change in CYP2E1 expression (data not shown).

The induction of CYP2E1 may be associated with the altered plasma glucose and glycine utilization (Son et al., 2000; Kim et al., 2001). Treatment with rGH to hypophysectomized rats resulted in no induction of CYP2E1, which accompanied decreased in the plasma glucose content and in the utilization of glucose by hepatocytes (Son et al., 2000). In the current study, we also determined the effect of glucose on the induction of CYP2E1 in the liver of ARF rat. Administration of glucose decreased the plasma urea nitrogen in ARF rats (i.e., 167 versus 137 mg/dl) and also partly reduced the extent of CYP2E1 induction. Hence, the CYP2E1 expression and plasma urea nitrogen were both affected by glucose administration. The relationship between glucose catabolism and urea production was further supported in part by the observation that the plasma glucose was decreased in rats perfused with urea (12% decrease) or L-arginine (27% decrease). Hence, the utilization of L-arginine and urea production in association with the catabolism of glucose played a crucial role in the induction of hepatic CYP2E1. Disturbances in these metabolic pathways would pathophysiologically affect the expression of CYP2E1 with a change in the mRNA level.

In the present study, the plasma insulin level was slightly increased by ARF, with concomitant induction of CYP2E1. Although the CYP2E1 induction by ARF was partially reversed by rGH or glucose, the plasma insulin was not changed (Table 1). Therefore, the level of plasma insulin appeared not to be related to the CYP2E1 induction by ARF. Aconitase activity, which represents mitochondrial function, was measured by the turnover of citrate to isocitrate as an index of cellular energy utilization. Aconitase activity in the livers of rats with ARF was slightly decreased compared with control (670 ± 46 and 552 ± 178 mU/g wet liver in control and ARF rats, respectively). rGH treatment caused an increase in aconitase activity in ARF rats (831 ± 185 mU/g wet liver; P < 0.01). It is likely that the induction of hepatic CYP2E1 by ARF is related with the urea cycle in mitochondria.

In conclusion, the present study demonstrated that CYP2E1 was induced by ARF and that urea and L-arginine were both involved in the increases in CYP2E1 protein and mRNA in the liver. Hepatic CYP2E1 induction may be accompanied by increases in urea and L-arginine catabolism.

References
Kim SG, Kim EJ, Kim YG, and Lee MG (2000) Evidence for elevation of cytochrome P450 2E1 (CYP2E1) induction with a change in the mRNA level. CYP2E1 INDUCTION BY UREA 745
TABLE 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham control</th>
<th>Urea-Perfused</th>
<th>L-Arginine-Perfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>6.2 ± 2.6</td>
<td>16 ± 11*</td>
<td>19 ± 11*</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.3 ± 0.1</td>
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<td>AST (U/l)</td>
<td>156 ± 92</td>
<td>143 ± 102</td>
<td>111 ± 8</td>
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<tr>
<td>ALT (U/l)</td>
<td>33 ± 6.6</td>
<td>39 ± 3.9</td>
<td>28 ± 8</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>124 ± 3.5</td>
<td>109 ± 12</td>
<td>90 ± 20</td>
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<tr>
<td>Insulin (μU/ml)</td>
<td>2.7 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>1.5 ± 0.6</td>
</tr>
</tbody>
</table>

* Rats were exposed to 25 mM of urea through portal vein for 24 h, and blood chemistry was monitored.
** Rats were exposed to 25 mM of L-arginine through portal vein for 24 h, and blood chemistry was monitored.
* P < 0.05, significant as compared to sham control.

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