Sympathetic vesicovascular reflex induced by acute urinary retention evokes proinflammatory and proapoptotic injury in rat liver

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Yu, Hong-Jeng, Bor-Ru Lin, Hsuan-Su Lee, Chia-Tung Shun, Chih-Ching Yang, Ting-Yu Lai, Chiang-Ting Chien, and Su-Ming Hsu. Sympathetic vesicovascular reflex induced by acute urinary retention evokes proinflammatory and proapoptotic injury in rat liver. Am J Physiol Renal Physiol 288: F1005–F1014, 2005—Increased hepatic sympathetic activity affects hepatic metabolism and hemodynamics and subsequently causes acute hepatic injury. We examined whether the vesicovascular reflex evoked by bladder overdistension could affect hepatic function, specifically reactive oxygen species (ROS)-induced inflammation and apoptosis, through activation of the hepatic sympathetic nerve. We evaluated the hepatic hemodynamics, hepatic sympathetic nervous activities, and cytometrygrams in anesthetized rats subjected to acute urinary retention. We used a chemiluminescence method, an in situ nitro blue tetrazolium perfusion technique, and a DNA fragmentation/apoptosis-related protein assay to demonstrate de novo and colocalize superoxide production and apoptosis formation in rat liver. Acute urinary retention increased the hepatic sympathetic-dependent vesicovascular reflex, which caused hepatic vasoconstriction/hypoxia and increased superoxide anion production from the perportal Kupffer cells and hepaticocytes, which were aggravated by the increase in volume and duration of urinary retention. The ROS-enhanced proinflammatory NF-κB, activator protein-1, and ICAM-1 expression also promoted proapoptotic mechanisms, including increases in the Bax/Bcl-2 ratio, CPP32 expression, poly-(ADP-ribose)-polymerase cleavages, and DNA fragmentation and apoptotic cells in the liver. The proinflammatory and proapoptotic mechanisms were significantly attenuated in rats treated with hepatic sympathetic nerve denervation or catechin (antioxidant) supplement. In conclusion, our results suggest that acute urine retention enhances hepatic sympathetic activity, which causes hepatic vasoconstriction and evokes proinflammatory and proapoptotic oxidative injury in the rat liver. Reduction of the hepatic sympathetic tone or antioxidant supplement significantly attenuates these injuries.

reactive oxygen species; apoptosis

URINARY RETENTION OCCURS FREQUENTLY in patients with bladder outlet obstruction or neurogenic voiding dysfunction. Acute urinary retention (AUR) with a volume beyond physiological tolerance (overdistension) can become a physiological or pathological stressor, resulting in bladder injury (40). In addition, excessive stimulation of mechanical afferents in the overdistended urinary bladder could evoke a sympathetic nerve-mediated vesicovascular reflex, which could lead to vasoconstriction and functional impairment of the heart and kidney (7, 23) and, likely, in other visceral organs. Consequently, hypertension, tachycardia, and abnormal renal function are commonly seen in patients with AUR.

Animal experiments have shown that increased hepatic sympathetic activity may cause hepatic injury through alterations of hepatic metabolism and hemodynamics (2, 16, 17, 25, 39) or through reactive oxygen species (ROS)-mediated hypoxia/reoxygenation oxidative injury (31). Activated Kupffer cells, infiltrated leukocytes, and/or resident cells could be sources of ROS generated in the liver and subject to oxidative injury (3, 18, 36, 37).

In the present study, we sought to examine for the first time whether an AUR-evoked vesicovascular reflex could activate hepatic sympathetic nerve activity and, thereby, affect hepatic function. We measured the hepatic hemodynamics and sympathetic nerve activity, and we examined the cellular events involved in ROS-induced inflammation and apoptosis in rat livers subjected to AUR. We also evaluated whether an antioxidant supplement and/or a reduction in hepatic sympathetic tone could effectively ameliorate the oxidative injury.

MATERIALS AND METHODS

Surgery and animal preparation. Female Wistar rats (200–250 g) were housed at the Experimental Animal Center, National Taiwan University, at a constant temperature and with a consistent light cycle (light from 0700 to 1800). The body temperature was kept at 36.5–37.0°C by an infrared light and was monitored with a rectal thermometer. Animal care and the experimental protocol were in accordance with the guidelines of the National Science Council of the Republic of China (NSC 1997). All efforts were made to minimize both animal suffering and the number of animals used throughout the experiment.

For experiments, the rats were anesthetized with subcutaneous urethane (1.2 g/kg). PE-50 catheters were placed in the left carotid artery for measurements of heart rate and arterial blood pressure (ABP) by an ADI system (PowerLab/16S, ADI Instruments, Castle Hill, Australia) with a transducer (P23 1D, Gould-Statham, Quincy, MA), and in the left femoral vein for administration of anesthetics when needed. In some animals, a PE-50 tube was inserted into the bile duct for bile collection. At the end of each experiment, the animals were killed with an intravenous potassium chloride injection.

Experimental model of AUR. We inserted a PE-50 tube into the bladder through the urethra and tied it in place with a ligature around the urethral meatus (7). The catheter was connected to a pressure transducer and an infusion pump (Infors, CH-4103, Bottmingen, Switzerland) via a T tube connector. The bladder volume was ini-
increased by a steady infusion of 0.9% saline (0.10 ml/min) via the infusion pump. Micturition volume was defined as the infused volume at which a micturition reflex was elicited (7). AUR was set by infusion of a one-fold (AUR$_{x1}$) or two-fold micturition volume (AUR$_{x2}$) of saline into the bladder and maintained for various time intervals (up to 6 h). The bladder was emptied by drainage of the fluid via the transurethral catheter.

**Measurement of hepatic PO$_2$ and hemodynamics.** We monitored hepatic microvascular blood flow and liver oxygenation in response to AUR at the same location as previously described by Jordan et al. (19). Fiber optic microprobes combining a laser-Doppler system, an oxygen sensor, and a thermocouple were inserted into the liver using a micromanipulator. Hepatic PO$_2$ and microcirculation measurements were performed at a depth of 2 mm from the liver surface. Data were collected continuously at a 10-s average value before, during, and after AUR stimulation and were recorded to disk with the use of a data-acquisition ADI system. PO$_2$ measurements are single-point measurements, and the volume sampled is confined to the sensor tip (230-400 mm diameter).

For other hemodynamics, a cannula (polyethylene, 0.58-mm ID, 0.97-mm OD) used for monitoring portal venous pressure (PVP) was inserted in the portal vein and secured with tissue adhesive (3M Vetbond; Animal Care Products, St. Paul, MN). PE-50 tubing of a bile duct cannula was placed for measurement of bile flow. Portal venous blood flow (PVBF) was measured by using an ultrasound flow probe (2SB1388, Transonic Systems) placed around the portal vein, was measured continuously with a T206 recording system (Transonic Systems), and was displayed on the ADI system. We calculated the mean hepatic microvascular resistance by using the following formula (40)

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\text{Hepatic microvascular resistance (HVR)} = \frac{\text{Mean portal venous pressure (mmHg)}}{\text{Mean portal venous blood flow (ml/min)}}
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**Hepatic sympathetic nervous activity recording.** We measured the hepatic sympathetic nervous activity, PVP, and PVBF in response to AUR in six rats. The techniques for isolating hepatic sympathetic nerves (41) and recording nervous activities were described previously (40). Hepatic nerves close to the liver were crushed for removal of possibleafferent activity, and the hepatic sympathetic nerve activity was depressed with intravenous norepinephrine (1 μg in 0.1 ml saline). Neural activity was displayed as voltage per second.

**Stimulation of hepatic sympathetic nerves.** For evaluating the effect of hepatic sympathetic activation on hepatic dynamics, one branch of hepatic sympathetic nerves was dissected for electrical stimulation. An electric current of square-wave pulses of 20 V with a pulse duration of 0.05 ms was applied with a stimulator (Grass S88, Quincy, MA) through a stimulus-isolation unit (Grass SIU5B) and a constant-current unit (Grass CCU1A). Different frequencies (1–50 Hz) were used for evaluating the activation of hepatic sympathetic nerves on PVBF. Each frequency of stimulation applied to the liver was performed for 2 min.

**Microsurgical sympathectomy of hepatic sympathetic nerves.** Denervation of hepatic sympathetic nerves was performed for verifying the neural effect on liver injury. The peritoneum was opened and the portal vein, hepatic artery, and bile duct were exposed. A 3-mm-wide region of tissue around the vessels and the bile duct was removed for eliminating sympathetic influence. Ligaments surrounding the liver were also eliminated for removal of sympathetic nerves entering via this route. We opened the abdomen and exposed the portal vein for 10 min for a sham operation.

**Hepatic myeloperoxidase activity and plasma aspartate aminotransferase.** Frozen liver samples were homogenized, and myeloperoxidase (MPO) activity was assayed with H$_2$O$_2$ and O-dianisidine, as previously described (38). The plasma aspartate aminotransferase (AST) level was determined by use of a commercially available analytic kit (Sigma, St. Louis, MO).

**In vivo and in vitro chemiluminescence recording of ROS activity.** The ROS generation in response to AUR and subsequent bladder emptying were measured from the liver surface and bile by a modified chemiluminescence detection method, as described previously (6).

The rat was maintained on a respirator and a circulating water pad at 37°C in a dark box with a shielded plate. Only the liver window was left unshielded and was positioned under a reflector, which reflected the photons from the exposed liver surface onto the detector area. The measurement of ROS from the liver was started by intravenous infusion of a superoxide anion probe, 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo-[1,2-a]-pyrazin-3-one-hydrochloride (MCLA; 0.2 mg·ml$^{-1}$·h$^{-1}$, TCI-Ace, Tokyo Kasei Kogyo, Tokyo, Japan), throughout the experiment by use of a Chemiluminescence Analyzing System (CLD-110, Tohoku Electronic, Sendai, Japan). The MCLA-enhanced ROS counts were continuously recorded every 10 s during the bladder-prefilling, urinary retention, and postemptying periods. The real-time displayed ROS signal was registered as the ROS level generated from the liver surface. A measurement of bile ROS was detected by a lucigenin-amplified method, as previously described (6).

To verify the specificity of ROS activity, we added superoxide dismutase (500 U/ml), a known scavenger for superoxide in the intravenous solution (6).

For evaluating the effects of antioxidants on oxidative injury, identical experiments were performed in five rats fed for 1 wk with decaffeinated green tea extract (50 mg/kg, Numen Biotech, Taipei, Taiwan), which was composed of various types of catechins (328 mg/g of epigallocatechin gallate, 152 mg/g of epicatechin gallate, 148 mg/g of gallochocin gallate, 132 mg/g of epicatechin, 108 mg/g of epigallocatechin, 104 mg/g of gallochocin, and 44 mg/g of catechin). The catechin extract (0.25 g) was dissolved in 500 ml of deionized distilled water every day. Each rat received restricted fresh drink (100 ml/kg body wt) daily provided at 6 PM in each cage by using sealed bottles for 1 wk. For consistent dosage of catechins, for some rats the rest of the tea drink was administered at 4 PM on the second day. The mean dosage of catechins was 50 ± 1 mg/kg body wt in this study.

**In situ demonstration of superoxide generation and apoptosis formation.** A nitroblue tetrazolium (NBT) perfusion method was used for localizing de novo ROS generation in the liver (6). Rats ($n=3$ in control, one-fold or two-fold micturition volume with hepatic sympathetic nerve intact/denervation or with or without catechin treatment) were killed either at the end of urinary retention or at different times after the bladder had been emptied. An 18-gauge needle connected to an infusion pump was inserted into the portal vein. The liver was perfused with 37°C Hanks’ balanced salt solution (flow rate, 10 ml/min; pH 7.4), and the perfusate was allowed to drain from the left atrium. Once the hepatic blood had been completely washed out, NBT (1 mg/ml) was added to the solution, and the liver was perfused for an additional 10 min at a flow rate of 5 ml/min. All unreacted NBT was removed from the liver by the perfusion with Hanks’ solution. The NBT-perfused liver was removed and fixed in zinc/formalin solution and processed for histological examination of formazan deposits. The value of blue NBT deposits/total section area was counted by Adobe Photoshop 7.0.1 image software analysis.

We used anti-4-hydroxy-2-nonenal (HNE) antiserum (Alpha Diagnosti International, San Antonio, TX) (14) and the terminal deoxyuridylate transferase-mediated nick-end labeling (TUNEL) method (6) to determine the presence and extent of apoptotic cells as evidence of oxidative stress. The tissue sections (5 μm) of the liver were prepared, deparaffinized, and stained by using the methyl green, 4-HNE-, and TUNEL-avidin-biotin-complex method. Twenty high-power (×400) fields were randomly selected for each liver section, and the value of apoptotic cells/apoptotic cells and methyl green-stained cells) was counted.

For hepatic macrophage (ED1) staining, the tissue sections were incubated overnight at 4°C with a mouse anti-rat antibody to ED1 (CD68, 1:200, Serotec, Sydney, NSW, Australia). A biotinylated secondary antibody (Dako, Botany, NSW, Australia) was then applied.
followed by streptavidin conjugated to horseradish peroxidase (Dako). The chromogen used was Dako Liquid diaminobenzene (DAB). Twenty high-power (×200) fields were randomly selected for each liver section, and the value of ED1-positive cells was counted.

**EMSA.** The nuclear extracts from 0.5 g of fresh liver samples were obtained with a nuclear extraction kit (Panomics, Redwood City, CA). Nine micrograms of nuclear extracts were analyzed by using a commercially available Electrophoretic-Mobility Shift Assay (EMSA, Panomics) for identifying NF-κB and activator protein-1 (AP-1), which interact with DNA. Two transcription factor probe sets (AP-1 and NF-κB) were used for the Panomics EMSA kits. The EMSA procedure was followed according to the manual, and final results were obtained by using a chemiluminescence imaging system.

**Immunoblot analysis of apoptosis-related proteins.** We measured the expression of ICAM-1 (8), Bax, Bcl-2, caspase 3, and poly(ADP-ribose) polymerase (PARP) (6) in the total protein from liver subjected to AUR/emptying injury.

Antibodies raised against ICAM-1 (R&D Systems, Minneapolis, MN), Bax (Chemicon, Temecula, CA), Bcl-2 (Transduction, Bluegrass, Lexington, KY), the activation fragments (32 kDa of proenzyme and 17 kDa of cleaved product) of caspase 3 (CPP32/Yama/Apopain; Upstate Biotechnology, Lake Placid, NY), PARP (Promega, Madison, WI), and β-actin (Sigma) were used. All of these antibodies cross-react with the respective rat antigens. Proteins on SDS-PAGE gels were transferred to nitrocellulose filters and stained as described (6).

**DNA ladder assay.** The method for DNA extraction and electrophoresis was described previously (6).

**Statistical analysis.** All values were expressed as means ± SE. Differences within groups were evaluated by paired t-test. One-way analysis of variance was used for examining differences among groups. Intergroup comparisons were made with Duncan’s multiple-range test. A P value of <0.05 was considered to indicate significance.

**RESULTS**

**AUR evoked a hepatic sympathetic nerve-mediated vesicovascular reflex.** In response to bladder overdistension, an increase in ABP, intravesical pressure (IVP), PVP, HVR, and a decrease in PVBF could be demonstrated (Fig. 1). The responses were more evident in AUR×2 bladders compared with AUR×1 bladders.

Our previous data demonstrated that bladder mechanoreceptor activation by overdistension enhanced adrenal and renal sympathetic nerve activity, leading to a systemic hypertensive effect (7). We therefore examined the bladder overdistension’s effect on hepatic sympathetic activity and hepatic hemodynamics. As shown in Fig. 2, the increase in bladder pressure (from 0 to 75 ± 5 mmHg) elicited by AUR×2 significantly increased hepatic sympathetic nerve activity (from 100 to 160–230%) and then elevated ABP, mean ABP, and heart rate, indicating that exacerbated vesicovascular reflex by AUR plays a role in hepatic hemodynamics.

Figure 3 demonstrates that a graded increase in the frequency of electric stimulation in hepatic sympathetic nerves reduced the PVBF and increased the ABP, PVP, and HVR in a frequency-dependent manner, suggesting an activation of hepatic sympathetic nerve-mediated vasopressor response could be also enhanced by different degrees of bladder overdistension stimulation (Fig. 1).

We further confirmed the neural influence on hepatic hemodynamics by an hepatic denervation technique. As shown in Fig. 4, in rats with intact hepatic nerves and with the bladder emptied, the baseline IVP was ~0 ± 0 mmHg, and the ABP (118 ± 10 mmHg), PVP (3.1 ± 0.3 mmHg), bile flow (0.52 ± 0.04 ml/h), hepatic oxygen tension (31 ± 2 mmHg), and PVBF (11.4 ± 1.9 ml/min) were maintained at stable levels. In response to AUR×2, the IVP was elevated to 32 ± 2 mmHg. The increased IVP triggered by bladder overdistension caused an elevation of the ABP by 26 ± 4 mmHg and of the PVP by 3.5 ± 0.7 mmHg. Meanwhile, a reduction in PVBF (decreased to 7.2 ± 1.1 ml/min at the end of AUR×2, and further decreased to 5.7 ± 0.9 ml/min at 1 h post-bladder emptying, P < 0.05) and in bile flow (from 5.4 ± 0.3 to 4.0 ± 0.3 ml/h) and an increase in the calculated HVR (175 ± 19% at the end of AUR×2 and 208 ± 26% at 1 h postemptying, P < 0.05) were found. In addition, AUR via vesicovascular reflex reduced hepatic PO2 and microvascular blood flow (Fig. 4). In rats subjected to denervation of the hepatic nerves, the effect of
enhanced vesicovascular reflexes on hepatic vasoconstriction and hypoxia/hypoperfusion was abolished.

AUR increased ROS amounts from the liver and bile. Our recent report suggested that hypoperfusion-hypoxia followed by reperfusion in the tissue could enhance ROS production, increase vascular resistance, and lead to subsequent injury (40). Additionally, the decreased bile outflow may evoke some oxidative stress in the rat liver. Therefore, we measured liver ROS directly by a modified chemiluminescence method (6, 7, 40). Intravenous infusion of MCLA led to a baseline level of ROS counts in a range of 1,900–2,000 counts (Fig. 5, A and B). On urinary retention, the value of ROS from the liver surface started to increase (usually at ~30 min after urinary retention) and was elevated to 4,300 ± 480 and 6,150 ± 560 counts at the end of overdistension and at 15 min after the bladder had been emptied, respectively. The hepatic ROS production was accompanied by increased ROS secretion in the bile (from 310 ± 29 counts in baseline value to 750 ± 60 counts at the end of the overdistension stage and 1,500 ± 120 counts at 15 min post-emptying; Fig. 5C). The livers with hepatic denervation failed

Fig. 2. Effects of AUR on ABP, mean arterial blood pressure (MBP), IVP, heart rate (HR), and hepatic sympathetic nervous activity (HSNA). A: an abrupt increase in IVP elicited a concurrent increase in HSNA, ABP, MBP, and HR. B: a slow tracing from A demonstrated that the increase in HSNA started ~5 s after an abrupt increase in IVP.

Fig. 3. A: effect of electrical stimulation of hepatic sympathetic nerves with different frequencies (in Hz) on the responses of ABP, PVP, PVBF, and HVR. B: level of increases in ABP (ΔABP), PVP (ΔPVP), and HVR (ΔHVR) and decrease in PVBF (ΔPVBF) by graded increases in stimulation (Hz) displays a frequency-dependent manner, indicating hepatic sympathetic activation can evoke a vasopressor response.
to exhibit similar increases in the ROS level in response to AUR, suggesting that the enhanced ROS is mediated by hepatic sympathetic nerves.

**AUR increased hepatic NBT deposits, 4-HNE stains, and apoptotic cell death.** We used an NBT and 4-HNE staining method for localizing the exact site of ROS generated in the liver. NBT is a dye that is reduced to an insoluble formazan derivative on exposure to superoxide (6). The blue of formazan is readily detectable in the tissue by light microscopy. NBT deposits (superoxide generation) did not appear in the livers of rats without AUR (Fig. 6, A, C, E, and H). There was a significant increase in superoxide production (blue formazan deposits) in the liver at 6 h post-bladder emptying from a 3-h AUR×2 liver (Fig. 6, B, D, F, and I). The NBT deposits were mainly found in the Kupffer cells (ED1 stain) located in the periportal region (Fig. 6, B and D), followed by the pericentral region. Some NBT deposits could be found in periporal hepatocytes (Fig. 6, B and D). 4-HNE stains (brown) also supported the blue NBT deposits in a similar location of AUR livers (Fig. 6F) but did not appear in the normal liver (Fig. 6E) or AUR liver with hepatic denervation (Fig. 6G). These data implied that the periportal areas are preferentially accumulated oxidative adducts and are possibly vulnerable to oxidative injury. ROS oxidized macromolecules and contributed to apoptosis (6, 7, 40). We performed double staining with the use of the NBT and TUNEL methods for evaluating the spatial relationship of ROS production and apoptotic nucleus formation in liver sections. The apoptotic cells were distinguished by

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**Fig. 4.** Mean values of ABP, PVP, PVBF, oxygen tension (PO2), hepatic microvascular blood flow (HMVBF), bile flow, and HVR at different times in response to AUR×2 for 180 min, and for a further 60 min after the bladder had been emptied, are shown. In rats (n = 6) with an intact hepatic sympathetic nerve (HI), AUR×2 significantly decreased PVBF, PO2, HMVBF, and bile flow and increased PVP and HVR. In hepatic sympathetic-denervated (HX) rats, the hepatic vasoconstrictor effect was significantly reduced. *P < 0.05 vs. control value (C). #P < 0.05 HI vs. HX.

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**Fig. 5.** Effects of AUR×2 on in vivo reactive oxygen species (ROS) generation in the livers of HI and HX rats. A: increase in hepatic ROS occurred in an HI rat but not in an HX rat. The increase in ROS became apparent 30 min after AUR. Emptying of the bladder further evoked a rapid increase in ROS in an HI rat, which reached a peak ~10–15 min after the bladder had been emptied. Mean values of hepatic ROS (B) and bile ROS (C) generation measured at different times during AUR in rats are depicted. *P < 0.05 vs. control (C). #P < 0.05 between HI (n = 10) and HX (n = 6).
their brownish-stained nuclei. Apoptotic cells were not detected in sections from the control rat liver (Fig. 6H). Both NBT deposits and apoptotic nuclei could readily be detected in both Kupffer cells and hepatocytes of perportal areas of AUR livers (Fig. 6I). In rat livers with hepatic denervation (Fig. 6, G and J) or catechin pretreatment (data not shown), a significant decrease in NBT deposits in the hepatocytes was observed. Increased volume in AUR increased the values of NBT deposits (4-HNE and NBT; Fig. 6K). NBT deposits, 4-HNE, and terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) stains for apoptotic cell death were absent in the control liver (A and C). NBT deposits, 4-HNE, and terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) stains for apoptotic nuclei in periportal hepatocytes in rats subjected to AUR, but less evident in the control liver (A and C). NBT deposits, 4-HNE, and terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) stains for apoptotic nuclei in periportal hepatocytes in rats subjected to AUR, but less evident in the control liver (A, E, and H). Double staining (NBT+TUNEL) was used to locate ROS-induced (NBT) apoptotic cells (arrows; i.e., cells with brownish-colored nuclei; I and J). Note the colocalization of NBT deposits and apoptotic nuclei in perportal hepatocytes in rats subjected to AUR (I and inset). Hepatic sympathetic denervation significantly reduced ROS production (4-HNE and NBT; G) and apoptosis formation (J). A and B: hematoxylin stain; C and D, NBT+ED1 counterstain; E–G, NBT+4-HNE counterstain; H–J, NBT+TUNEL counterstain. Mean values of NBT deposits (n = 4 in each group) and TUNEL stain (n = 4 in each group) are displayed in K and L.

Effect of AUR on proinflammatory and apoptosis-regulated gene expression. ROS triggered early cellular signal transduction pathways responsible for the activation of NF-κB and AP-1, resulting in upregulation of the ICAM-1 gene in the insulted tissue (4, 28). The proinflammatory response of NF-κB and AP-1 translocation and ICAM-1 protein expression in the livers subjected to AURx1 or AURx2 is displayed in Fig. 7. Significant hepatic nuclear binding of transcription factors NF-κB and AP-1 was observed in the AURx2 liver with different time frames of emptying (Fig. 7A). The enhanced hepatic NF-κB and AP-1 binding activity was partly inhibited by hepatic denervation or green tea treatment (Fig. 7B). ICAM-1 expression was increased during urinary retention and was further enhanced after the bladder had been emptied (Fig. 7C). Increases in the volume and interval of urinary retention, as well as the interval after bladder emptying (traced up to 6 h), significantly potentiated the proinflammatory responses in the insulted liver. The expression of hepatic MPO activity also increased in response to urinary retention (Fig. 7D). The increased proinflammatory responses were inhibited by hepatic sympathetic denervation or pretreatment with catechins. A significant increase in ED1-positive hepatic macrophage number was found in the 3-h AURx2 liver after 6 h of emptying (215 ± 29 counts/HPF, Fig. 7F) but was less evident in the normal liver (65 ± 8 counts/HPF, Fig. 7E) or 3-h AURx2 denervated liver after 6 h of emptying (98 ± 10 counts/HPF, Fig. 7G), suggesting possible oxidants come from hepatic macrophages.

Ischemia-reperfusion or hypoxia-reoxygenation-induced ROS contributed to apoptotic cell death (6, 7, 40). The expression of Bax, Bcl-2, CPP32, and PARP in the liver after AUR/emptying injury was also assessed by immunoblotting with antibodies against Bax, Bcl-2, CPP32, and PARP (Fig. 8A). The Bax/Bcl-2 ratio and the expression of 32 kDa (proenzyme) and 17 kDa (cleaved product) of CPP32 and PARP were all increased in livers subjected to urinary retention. Similar to the proinflammatory response, the proapoptotic signaling pathway and AST level (Fig. 8B) were also enhanced by the distension volume, the duration of distension, as well as the interval after bladder emptying. The enhanced proinflammatory and proapoptotic expression and AST level could be suppressed by hepatic denervation or treatment with catechins (Fig. 8). By pathological evaluation, we found that a marked increase in perportal congestion was observed in the 3-h AURx2 liver after 24 h of...
emptying (Fig. 8, D and F) compared with the normal liver (Fig. 8, C and E).

Effects of bladder overdistension on DNA fragmentation patterns in the rat liver. By gel electrophoresis, no apparent DNA fragmentation was detected from livers subjected to AUR. An apoptotic DNA laddering pattern was clearly evident in livers 4–6 h after the bladder had been emptied after a 3-h overdistension (data not shown). The DNA laddering pattern was attenuated in rats treated with HSN denervation or with catechin supplement.

DISCUSSION

Substantial anatomic and physiological evidence has suggested a role of hepatic sympathetic nerves in the regulation of hepatic function (1, 2, 20, 22, 41). Continuous and repeated activation of hepatic sympathetic activity by physiological stressors and electrical stimulation caused hepatic vasoconstriction and hypoxia-reoxygenation, resulting in hepatic injury (16, 17). In the present study, we have demonstrated that AUR could reduce the hepatic blood flow and oxygenation via activation of the hepatic sympathetic nerve, and, after the bladder had been emptied, restoration of hepatic blood flow and oxygenation could enhance ROS generation/secretion, the proinflammatory response, and cascades of cellular apoptosis in the rat liver. These were demonstrated by enhancement of hepatic MPO activity with increased volume and interval of AUR and was further enhanced after the bladder had been emptied. The increased expression of these specific proteins were significantly reduced by HX or 1-wk GT treatment (50 mg·kg⁻¹·day⁻¹). Equal protein loading was displayed by β-actin. D: enhanced expression of hepatic MPO activity with increased volume and interval of AUR and interval post-bladder emptying were also significantly reduced by HX or 1-wk GT treatment. Increased expression of ED1-stained hepatic macrophages is found in the AUR liver after 6 h of emptying (F) compared with normal (E) or AUR liver with HX treatment (G). *P < 0.05 vs. control. #P < 0.05 vs. 3 h of AUR and 6-h emptying without any treatment.
We used an enhanced chemiluminescence method to study ROS, especially O$_2^-$ production in the liver and bile. This method has been well established for measurement of ROS production in cultured cells, the whole blood system, isolated perfused organs, urinary bladder, and the kidney in vivo (6, 8). We showed that the ROS level detected from the liver surface and secretion of ROS in bile increased 30 min after AUR and that this ROS value was further enhanced after the bladder was emptied. Our results are similar to the data reported by Suematsu et al. (32) showing that, in the liver subjected to low-flow hypoxia, superoxide-dependent oxidative stress and resultant cell death occurred as early as 20 min after hypoxia and reached a peak at 60 min. By employing an in situ vascular NBT perfusion technique, we showed that the cellular source of ROS, especially O$_2^-$ synthesis, was located in Kupffer cells and hepatocytes, mainly around the periportal regions and, to a lesser degree, in the midzonal and pericentral regions of the insulted liver. The primary location in the periportal area may be ascribed to maximal intercellular propagation of the norepinephrine signal from the periportal (upstream) area, where it is received from sympathetic nerve endings, to perivenous (downstream) hepatocytes (1, 2, 17, 20, 22). It has been reported that oxidative adducts preferentially accumulated in periportal hepatocytes, endothelial cells, and Kupffer cells and preceded cell death in hypoperfused rat liver (15, 32). On the other hand, the periportal area is more highly supplied by PO$_2$ than other areas; therefore, the periportal area is vulnerable to hypoperfusion-reperfusion-like injury.

Kupffer cells and the resident macrophages in the liver play a critical role in the pathogenesis of several inflammatory diseases (15, 36), such as alcohol liver disease (15, 26) and liver ischemia-reperfusion (3, 37), which frequently lead to localized tissue injury. Under hypoxic (vasoconstriction) conditions, Kupffer cells may be activated and produce superoxide after tissue reperfusion (11, 15, 29, 35). On the other hand, oxidative stress may lead to vasoconstriction and disturbance of the hepatic microcirculation and further aggravate liver injury (21, 34). In the present study, we demonstrated that Kupffer cells also played a vital role in hepatic sympathetic nerve-mediated oxidative injury in the livers of rats subjected to the bladder overdistension/emptying-induced hypoperfusion-reperfusion insult. The above effects may be minimized in
rats pretreated with antioxidants. Our result is similar to that in a recent study by Froh et al. (13), in which the injection of transducer Kupffer cells encoding the Cu/Zn-SOD gene significantly blunted the increase in the serum AST level and tissue injury in the liver subjected to ischemia-reperfusion injury.

In organs subjected to septic shock, hemorrhage, and ischemia-reperfusion, the overproduced ROS triggered early cellular signal transduction pathways responsible for the activation of NF-κB and AP-1, resulting in upregulation of the ICAM-1 gene in the vascular endothelium and subsequent tissue accumulation of activated neutrophil accumulation (4, 28). Similarly, we demonstrated that the hypoxia-reoxygenation-like hepatic injury initiated by bladder overdistension/emptying also evoked a burst in the release of ROS that led to early activation of nuclear translocation of the p65 subunit of NF-κB and AP-1, which, in turn, promotes the expression of ICAM-1 protein. This proinflammatory response can be abrogated by catechins, which exert antioxidant (scavenging ROS activity) and anti-inflammatory activity (decreasing NF-κB, AP-1, and ICAM-1) on AUR-enhanced oxidative stress.

In addition to the proinflammatory response, the overproduced ROS in the cells under hypoxia-reoxygenation conditions may be responsible for the apoptotic death of these cells (6). Our study showed that prolonged bladder overdistension and subsequent emptying resulting in varying degrees of hepatocellular apoptosis in a time- and volume-dependent manner. This was demonstrated by increases in DNA fragmentation, in apoptotic cell number, and in “proapoptotic” proteins such as Bax, CPP32, and PARP. A minimum AUR for 3 h followed by 4 h post-bladder emptying is required for the detection of apoptotic nuclei in the liver. Our study shows that administration of catechins inhibited ROS production (data not shown), NF-κB and AP-1 activation, reduced the expression of ICAM-1 at protein levels, and attenuated hepatocellular apoptosis in the hypoperfused/reperfused livers. Catechins with antioxidant, antiapoptotic, and anti-inflammatory activity can inhibit redox-sensitive transcription factors in cancer cells (9, 27), in hepatic stellate cells in hepatic fibrosis (5), and in insults to cells or tissues mediated by ROS (4, 9, 24, 27, 28, 30).

Effective scavenging of ROS or maintenance of the cellular redox state may represent a useful therapeutic approach for limiting inflammation- and apoptosis-mediated hepatic reperfusion injury.

A previous study in humans demonstrated by Lee et al. (23) has indicated that a distension of the urinary bladder, a physiological stimulus, could induce impaired coronary circulation in patients with early atherosclerosis via sympathetic activation on α1-adrenergic receptor. This important impact may imply a similar influence on hepatic vasoconstriction despite a different innervation of the sympathetic nerve in rat and human livers. To the best of our knowledge, AUR-induced hepatic injury has not been well recognized in the clinical setting. Two explanations may be responsible for this. First, in treating patients with AUR, physicians focus on the functional recovery of the urinary bladder, kidney, and cardiovascular system. Second, a minor hepatic injury is hardly recognized in the clinical setting, and it is likely that the oxidative injury is repaired in a short time by the scavengers and/or regeneration of hepatocytes. We have found a reduction of hepatic blood flow by Duplex ultrasound in some volunteers in the condition of urgency to urinate (Lee H.-S., Yu H.-J., and Chien C.-T., unpublished observations). Thus it is of interest to examine whether differential responses will occur in patients who already have impaired hepatic function. Additionally, a long-term complete or partial bladder outlet obstruction may have a detrimental effect on hepatic function.

In summary, the present study indicates that ROS are produced by the peritoneal Kupffer cells and hepatocytes in response to prolonged bladder overdistension through the sympathetic nerve-mediated vesicovascular reflex, which triggers proinflammatory and proapoptotic signaling pathways and may therefore cause hepatic injury. Hepatic sympathetic denervation or treatment with free radical scavengers appears to prevent this ROS-mediated hepatic injury.

GRANTS

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ACUTE URINARY RETENTION-INDUCED LIVER INJURY


