Norepinephrine-induced hepatocellular dysfunction in early sepsis is mediated by activation of $\alpha_2$-adrenoceptors

SHAOLONG YANG, MIAN ZHOU, IRSHAD H. CHAUDRY, AND PING WANG

Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama 35294

Received 8 February 2001; accepted in final form 22 May 2001

Gut-derived norepinephrine (NE) has been shown to play a critical role in producing hepatocellular dysfunction in early sepsis, but it is not known whether $\alpha_2$-adrenoceptor activation mediates this dysfunction. We infused normal male adult rats with NE, NE plus the specific $\alpha_2$-adrenergic antagonist rauwolscine (RW), or vehicle (normal saline) for 2 h. Hepatocellular function was determined by in vivo indocyanine green (ICG) clearance. An isolated perfused liver preparation was also used to assess hepatocellular function by in vitro ICG clearance; NE alone or with RW was added to the perfusate. Rats were subjected to sepsis by cecal ligation and puncture (CLP). At 1 h after CLP, RW was infused for 15 min. At 5 h after CLP, we measured hepatocellular function and serum tumor necrosis factor-$\alpha$ (TNF-$\alpha$) levels. Intraportal NE infusion in normal rats produced hepatocellular dysfunction, which was prevented by RW and NE infusion. This is confirmed by findings with the isolated perfused liver preparation. RW administration in early sepsis maintained hepatocellular function and downregulated TNF-$\alpha$ production at 5 h after CLP. These results suggest that NE-induced hepatocellular dysfunction in early sepsis is mediated by $\alpha_2$-adrenoceptor activation, which appears to upregulate TNF-$\alpha$ production. Modulation of hepatic responsiveness to NE by $\alpha_2$-adrenergic antagonists should provide a novel approach for maintaining cell and organ functions during sepsis.

BECAUSE OF ITS IMPORTANT ROLE IN METABOLISM AND HOST DEFENSE MECHANISMS, THE LIVER HAS BEEN Extensively studied during sepsis and septic shock and is thought to be a major organ in the development of multiple organ failure under such conditions (41). Although hepatic failure is generally thought to be a late complication following pulmonary and renal failures (2), previous studies (34, 36) have shown that hepatocellular dysfunction occurs very early after the onset of sepsis and that the depression of hepatocellular function does not appear to be due to a reduction in hepatic perfusion in early sepsis. It is therefore important to determine the factor(s) released early after the onset of sepsis) that might be responsible for producing hepatocellular dysfunction. In this regard, proinflammatory cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) have been implicated (31, 32) as important mediators responsible for producing cellular dysfunction and metabolic alterations during sepsis. Kupffer cells are known to be a major source of proinflammatory cytokine release during sepsis as well as under other adverse circulatory conditions (5, 16, 26). It has been suggested (25) that the gut may be the “motor” for initiating multiple organ dysfunction after injury. In this regard, we (49) have recently demonstrated that the gut becomes a significant source of norepinephrine (NE) release during sepsis and gut-derived NE plays a crucial role in depressing hepatocellular function during the early stage of sepsis. Moreover, Spengler et al. (28, 29) reported that stimulation of peritoneal macrophages with NE augments TNF-$\alpha$ production, which appears to be mediated by an $\alpha$-adrenergic mechanism. Based on these observations, we have postulated that gut-derived NE produces hepatocellular dysfunction during sepsis via its upregulatory effect on TNF-$\alpha$ production by Kupffer cells, which is mediated by the activation of $\alpha_2$-adrenoceptors in the liver (17). Although perfusion of the isolated liver with NE, at a concentration similar to that observed during sepsis (10), produces hepatocellular dysfunction, it remains unknown whether or not hepatocellular dysfunction observed under such conditions is mediated at least in part by the activation of $\alpha_2$-adrenoceptors. The objectives of this study therefore were to determine 1) whether coadministration of the specific $\alpha_2$-adrenergic antagonist rauwolscine (RW) prevents NE-induced hepatocellular dysfunction and 2) whether administration of RW early after the onset of sepsis maintains hepatocellular function and downregulates the proinflammatory cytokine TNF-$\alpha$.

MATERIALS AND METHODS

Experimental animals. Adult male Sprague-Dawley rats (275–325 g), purchased from Charles River Laboratories.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
α₂-ADRENOCEPTORS AND THE LIVER IN SEPSIS

(Wilmington, MA), were used in the present study. All surgery was performed using aseptic procedures with the exception of the induction of sepsis by cecal ligation and puncture (CLP). The experiments described below were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892]. This project was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Intraportal infusion of NE or NE plus RW in normal animals. Rats were fasted overnight but allowed water ad libitum before the experiment. The animals were anesthetized with isoflurane inhalation during the cannulation of a femoral vein, and the anesthesia was maintained by intravenous pentobarbital sodium (30 mg/kg body wt) during the entire experimental period. A 4-cm midline incision was performed, and a branch of the superior mesenteric vein was isolated and cannulated with PE-10 tubing (Becton Dickenson, Parsippany, NJ). The tip of the catheter was advanced toward the liver, reaching the portal vein. After the catheter was secured, the abdominal wall was closed in layers. The left femoral artery was also cannulated with PE-50 tubing and connected to a blood pressure analyzer (Micro-Med, Louisville, KY) to monitor the mean arterial pressure (MAP) and heart rate during the infusion of various agents. NE (20 μM, Sigma, St. Louis, MO) or NE (20 μM) and the specific α₂-adrenergic antagonist RW (1 mM, Tocris, Ballwin, MO) were infused via the portal catheter by a Harvard pump. The infusion rate was set at 13 μl/min, and the total infusion time was 2 h. Control animals were infused only with normal saline via the portal vein at a rate of 13 μl/min for 2 h. Portal blood flow was reported to be 13 ml·min⁻¹·liver⁻¹ in the rat (35). Thus infusion of 20 μM NE solution at a rate of 13 μl/min would increase portal blood levels of NE to ~20 nM, which is similar to that observed during sepsis (10, 49). This concentration of NE (~20 nM) was used because our recent study indicated that 20 nM NE reduces hepatocellular function using an isolated perfused liver preparation. The dose of RW used in this study was 50-fold higher than NE to completely block the α₂-adrenergic effects of this catecholamine.

Determination of hepatocellular function and cardiac output. Hepatocellular function was determined using an in vivo indocyanine green (ICG) clearance technique at the completion of the 2-h NE infusion as we (34) previously described in detail. In brief, rats were anesthetized with isoflurane inhalation, and a 2-French fiberoptic catheter was inserted to the level of the aortic arch via the right carotid artery. Three doses of ICG (0.167, 0.333, and 0.833 mg/kg body wt) were administered via the jugular vein catheter, and the ICG concentration in the circulation was recorded each second for 5 min using an in vivo hemofloimeter and computer-assisted data acquisition. An e⁻ raised to a second-order polynomial (ICG = a₀ + b_x + c_x²) was used to determine the initial velocity of ICG clearance. The maximal velocity of the ICG clearance (V_max) and the Michaelis constant (K_m) were determined from the Lineweaver-Burk plot (41, 43). It should be noted that V_max represents the number of functional ICG carriers or transporters of the active hepatocellular ICG transport system and K_m represents the efficiency of the active transport process (41). Cardiac output was determined by using a dye dilution technique immediately at the end of the 2-h infusion as previously described (34).

Isolated perfused liver preparation and in vitro ICG clearance. An isolated perfused rat liver preparation was used as described in detail previously (48, 49). In brief, the anesthetized rats underwent a longitudinal midline incision and transverse subcostal incision. The common bile duct was cannulated with a PE-10 catheter for collecting the bile. The distal vena cava and distal portal vein were ligated with sutures. The portal vein was then immediately cannulated with a 16-gauge silicon catheter, and perfusion was initiated within 1 min. The catheter was connected to a three-limb tube that was attached to a perfusion pump and to a syringe filled with 3 ml normal saline containing heparin (20 U/ml). A blood pressure analyzer was connected to the portal catheter using PE-50 tubing to monitor the portal venous perfusion pressure. While the portal catheter was secured, 3 ml heparin saline were injected into the portal vein. This was immediately followed by perfusion with Krebs-Henseleit buffer with 0.1% glucose and 0.5% BSA (fraction V) gassed with 95% O₂-5% CO₂ at a consistent rate of 35 ml/min for 60 min (49). NE (20 nM) alone or plus RW (1 μM) was added to the perfusate and present throughout the entire period of perfusion. Before the determination of in vitro ICG clearance (a measure of hepatocellular function), the isolated liver was perfused with Krebs-Henseleit buffer for 30 min without recirculation and then perfused with an additional 400 ml of perfusate containing 8 μg ICG with recirculation. Samples of effluent (1 ml each) were collected every 5 min for 30 min after ICG administration. The ICG concentration in the effluent was determined using a spectrophotometer at a wavelength of 800 nm (35). The difference in ICG content between different time points was the amount of ICG taken up by the liver. At the end of the experiment, the livers were harvested for determination of dry weight, and hepatic ICG clearance was expressed as micrograms per gram of dry liver.

Animal model of sepsis. Polymicrobial sepsis was induced by CLP as previously described (6, 42). In brief, male adult Sprague-Dawley rats were fasted overnight but allowed water ad libitum before the experiment. The animals were then anesthetized with isoflurane inhalation, and a 2-cm midline incision was performed. The cecum was exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, and then punctured twice with an 18-gauge needle. The puncture was squeezed to expel a small amount of fecal material, and the abdominal incision was closed in two layers. Sham-operated rats underwent the same surgical procedure except that the cecum was neither ligated nor punctured. All animals received normal saline (3 ml/100 g body wt) subcutaneously immediately after the operation to provide fluid resuscitation. At 1 h after CLP, RW at 1 mg/kg body wt (9) in 1 ml normal saline or 1 ml normal saline alone was infused via the femoral venous catheter over 15 min. In addition, a femoral artery was also cannulated with PE-50 tubing, which was connected with a blood pressure analyzer to monitor MAP and heart rate during RW infusion. RW administration did not significantly alter MAP or heart rate (data not shown). At 5 h after CLP, hepatocellular function and serum levels of TNF-α were determined.

Assay of serum TNF-α. After the determination of hepatocellular function, blood samples (~1 ml) were collected by cardiac puncture. The samples were put on ice for 10 min and then centrifuged at 1,200 g for 10 min, and the serum was stored at ~70°C until assayed. The serum level of TNF-α was measured using an ELISA kit (BioSource International, Camarillo, CA) according to the manufacturer's suggested protocol.

Statistical analysis. Data are presented as means ± SE. One-way ANOVA and Tukey’s test were employed for comparison among different groups of animals. Differences were considered significant at P ≤ 0.05.
RESULTS

Effects of intraportal administration of NE or NE plus RW on hepatocellular function in normal animals. As indicated in Fig. 1, \( V_{\text{max}} \) and \( K_m \) decreased by 44% (\( P < 0.05 \)) and 48% (\( P < 0.05 \)), respectively, at the end of the 2-h intraportal infusion of 20 \( \mu \)M NE compared with vehicle (normal saline)-infused animals. However, infusion of NE (20 \( \mu \)M) in combination with RW (1 mM) maintained hepatocellular function. \( V_{\text{max}} \) and \( K_m \) increased by 61% (\( P < 0.05 \)) and 65% (\( P < 0.05 \)), respectively, at the completion of the 2-h infusion of NE plus RW compared with NE-infused animals (Fig. 1). The effects of NE or NE plus RW on hepatocellular function were not due to changes in systemic hemodynamic parameters, because cardiac output was not altered under such conditions (37.4 ± 0.6, 37 ± 0.4, and 37.2 ± 0.5 ml min \(^{-1} \)·100 g body wt \(^{-1} \) in normal saline-, NE-, or NE plus RW-infused animals, respectively; \( n = 6 \) group). Similarly, both MAP and heart rate did not change significantly after intraportal administration of NE or NE plus RW (data not shown).

Effects of NE or NE plus RW on hepatocellular function using an isolated perfused liver preparation from normal animals. As shown in Fig. 2, the perfused livers isolated from normal animals demonstrated effective ICG clearance. The addition of 20 nM NE to the perfusate, however, significantly reduced ICG clearance at 40–60 min after its addition (by 44–48%, \( P < 0.05 \)). The decreased ICG clearance induced by NE was attenuated by coadministration of 1 mM RW. There was no significant difference in ICG clearance between vehicle and NE plus RW groups (Fig. 2). Additionally, there was no significant difference in bile production among livers perfused with vehicle, NE, or NE plus RW (Fig. 3), indicating that the viability of the liver was not altered by administration of NE or RW.

Effects of RW on hepatocellular function and circulating levels of TNF-\( \alpha \) during early sepsis. At 5 h after the onset of sepsis (i.e., the early stage of polymicrobial sepsis; 41), \( V_{\text{max}} \) decreased by 57% compared with sham-operated animals (\( P < 0.05 \), Fig. 4A). Intravenous administration of RW at 1 h after CLP, however, increased \( V_{\text{max}} \) by 107% (\( P < 0.05 \)) compared with septic animals treated with vehicle (normal saline) and was not different from sham-operated animals (Fig. 4A). Similarly, \( K_m \) decreased by 57% at 5 h after CLP compared with sham-operated animals, and administration of RW attenuated the decrease in \( K_m \) (Fig. 4B). As indicated in Fig. 5, circulating levels of TNF-\( \alpha \) increased approximately fourfold at 5 h after the onset of sepsis (i.e., the early stage of polymicrobial sepsis; 41).
of sepsis \((P < 0.05)\). Administration of RW at 1 h after CLP, however, reduced the serum levels of TNF-\(\alpha\) by 68\% \((P < 0.05)\), and the levels were similar to the sham group (Fig. 5).

**DISCUSSION**

Studies (34, 36, 41) have shown that hepatocellular function, as assessed by in vivo ICG clearance, is depressed early after the onset of sepsis and is further reduced with the progression of sepsis. The depression of hepatocellular function in early sepsis does not appear to be due to any reduction in regional perfusion, because total hepatic blood flow increases significantly under those conditions (35, 36). Thus it appears that hepatocellular dysfunction observed during early sepsis may be due to factors other than a disturbance in hepatic perfusion. In this regard, the proinflammatory cytokine TNF-\(\alpha\) has been suggested (37, 41) to play an important role in producing hepatocellular dysfunction. Although it has been shown that the gut is capable of producing proinflammatory cytokines after hemorrhagic shock or systemic inflammation (7, 15, 24), our (19) recent studies have indicated that the gut does not appear to contribute to the elevated levels of proinflammatory cytokines, e.g., TNF-\(\alpha\), interleukin-1\(\beta\) (IL-1\(\beta\)), and IL-6, observed during sepsis. Several lines of evidence (15, 17, 27, 32) suggest that the activation of Kupffer cells is responsible for proinflammatory cytokine release and subsequently hepatocellular dysfunction during sepsis. Our (16) recent studies suggest that Kupffer cells are the major source of proinflammatory cytokines such as TNF-\(\alpha\), IL-1\(\beta\) and IL-6 in early sepsis. The number of Kupffer cells in male adult rats was reduced in vivo by intravenous injection of gadolinium chloride (GdCl\(_3\); 10 mg/kg) 48 h before CLP (i.e., an animal model of polymicrobial sepsis). The results indicate that the circulating levels of TNF-\(\alpha\) increased from nondetectible in sham-operated animals to 148.9 \(\pm\) 30.3 pg/ml at 5 h after CLP. Prior administration of GdCl\(_3\), however, significantly attenuated the increase in circulating TNF-\(\alpha\) levels to 35.1 \(\pm\) 20.2 pg/ml \((P < 0.05, n = 5/\text{group})\). Moreover, both IL-1\(\beta\) and IL-6 levels increased significantly at 5 h after CLP.
in Kupffer cell-intact animals, and Kupffer cell reduction by GdCl₃ significantly lowered circulating levels of IL-1β and IL-6 (16). These results indicate that Kupffer cells are indeed responsible for producing proinflammatory cytokines in early sepsis. Under septic conditions, there is an increase in peripheral sympathetic nerve activity and peripheral adrenergic stimulation, resulting in elevated plasma levels of catecholamines. In support of this, studies (10, 21) have indicated that plasma levels of NE increased significantly as early as 30 min after the onset of sepsis. Additionally, mesenteric organs (primarily the gut) have been demonstrated (1, 8, 49) to contribute substantially to total body NE production under normal conditions as well as during sepsis. Because of the intimate connection of the gut and liver via portal circulation, it is important to examine whether gut-derived NE affects hepatocellular function during sepsis. To this end, our (49) recent studies have indicated that gut-derived NE plays an important role in depressing hepatocellular function in the early stage of sepsis. Because stimulation of macrophage α-adrenoceptors appears to contribute to the production of TNF-α in vitro (28, 29), we hypothesized that hepatocellular dysfunction observed during the early stage of sepsis is mediated by the activation of α₂-adrenoceptors in the liver (presumably on Kupffer cells). The present study was therefore conducted to test this hypothesis and to determine whether modulation of hepatic responsiveness to NE stimulation by the specific α₂-adrenergic antagonist RW attenuates hepatocellular dysfunction in early sepsis.

Our results indicate that intraportal infusion of NE in normal animals for 2 h produced hepatocellular dysfunction. In addition, perfusion of NE in the isolated livers resulted in a significant decrease in ICG clearance. Administration of NE in combination with the specific α₂-adrenergic antagonist RW, however, prevented NE-induced decrease in ICG clearance during in vivo and in vitro conditions. The studies by Fessler et al. (9) have also shown that pretreatment with RW significantly reduced intestinal and hepatic injury as well as TNF-α levels after endotoxin shock. In addition, Kotanidou et al. (20) reported that administration of another α₂-adrenergic antagonist, urethane, significantly improved the survival rate and reduced TNF-α release after lethal endotoxemia. Our present data also demonstrated that intravenous administration of RW early after the onset of sepsis attenuated sepsis-induced hepatocellular dysfunction, which was associated with downregulation of the proinflammatory cytokine TNF-α. These results, taken together, would suggest that NE-induced hepatocellular dysfunction during sepsis appears to be mediated by the activation of α₂-adrenoceptors in the liver (presumably on Kupffer cells).

It should be noted that we did not directly determine the specific role of Kupffer cell α₂-adrenoceptors in upregulating TNF-α in the present study. However, our recent results have indicated that NE increases TNF-α release in cultured Kupffer cells as well as in isolated perfused liver preparation, which can be blocked by a specific α₂-adrenergic antagonist (50). In addition, incubation of Kupffer cells with the α₂-adrenergic agonist clonidine upregulates TNF-α in the cell culture system. Thus NE-induced release of TNF-α from Kupffer cells is mediated by the activation of α₂-adrenoceptors (50). Furthermore, the gut appears to be the major source of NE release during sepsis (49). With regard to the source of TNF-α in sepsis, our studies have indicated that Kupffer cells are responsible for the release of TNF-α (50) as well as other proinflammatory cytokines, such as IL-1β and IL-6 (16), during the early stage of sepsis. Moreover, studies from our laboratory (41) have demonstrated that TNF-α is indeed responsible for producing hepatocellular dysfunction observed during the early stage of sepsis. These results along with the findings in the present study suggest that gut-derived NE produces hepatocellular dysfunction during sepsis via its up-regulatory effect on TNF-α production by Kupffer cells, which is mediated by the activation of α₂-adrenoceptors. It could be argued that NE-induced upregulation of TNF-α may be due to an increase in endotoxin. This does not appear to be the case, because our (50) recent studies have indicated that intraportal administration of NE did not significantly alter plasma levels of endotoxin. This would suggest that NE-induced upregulation of TNF-α is endotoxin independent.

In the present study, ICG clearance was used as a measure of hepatocellular function because it is an extremely sensitive and early indicator of alterations in hepatocellular function in sepsis (41, 42). This technique is distinct from assessment of plasma liver enzymes, as it measures hepatocellular function, rather than injury, and thus reflects hepatocellular dysfunction as opposed to hepatocellular damage. We (36, 39) have reported that hepatic clearance of this dye decreased at 1.5 h, whereas circulating levels of liver transaminases (alanine aminotransferase and aspartate aminotransferase) did not increase until 10 h after the onset of sepsis. In addition, a cytosolic liver enzyme, α-glutathione S-transferase, a sensitive indicator of alterations of hepatocyte integrity, increased only as early as 5 h after CLP (18). Although sepsis-induced hepatocellular dysfunction appears to be due to multiple mediators, our (41) findings have suggested that upregulation of TNF-α plays an important role in depressing hepatocellular function under such conditions. In this regard, administration of a low dose of TNF-α, which does not alter cardiac output and hepatic perfusion, produced hepatocellular dysfunction (32). In addition, administration of pentoxifylline, which has been shown to downregulate TNF-α (30), maintained hepatocellular function at both 2 and 5 h after the onset of sepsis (38). Moreover, TNF-α is upregulated in Kupffer cells before the occurrence of hepatocellular dysfunction after the onset of sepsis (37). These results, taken together, suggest that TNF-α does play a major role in producing hepatocellular dysfunction after the onset of sepsis. Although we only determined serum levels of TNF-α at 5 h after CLP in the present study,
we (19, 41) have previously reported that this proinflammatory cytokine increased at various time points after CLP. However, it remains unknown whether administration of RW at 1 h after CLP reduces TNF-α levels at 20 h after the onset of sepsis. In a separate study (50), we have reported that administration of NE via the portal vein in normal animals and in the isolated perfused liver significantly increased TNF-α production, which was prevented by coadministration of the α2-adrenergic antagonist yohimbine. Studies (28) have demonstrated that α2-adrenergic agonists NE and UK-14304 enhanced endotoxin-stimulated TNF-α production by peritoneal macrophages. At the transcriptional level, α2-adrenergic agonists increase TNF-α mRNA accumulation, which can be blocked by the α2-adrenergic antagonist yohimbine (28). In support of these findings, our studies indicate that intraperitoneal administration of NE upregulated TNF-α gene expression in Kupffer cells as well as cellular and plasma levels of this cytokine, while coadministration of NE and the α2-adrenergic antagonist yohimbine prevented the increase in cellular and plasma TNF-α levels (50). Studies (14, 28) have also shown that TNF-α mRNA induced by α2-adrenergic agonists in peritoneal macrophages is endotoxin dependent. However, we have shown that stimulation of Kupffer cell α2-adrenoceptors by NE or another α2-adrenergic agonist, clonidine, without the presence of endotoxin also upregulates TNF-α production in a Kupffer cell culture system (50). The fact that α2-adrenergic antagonists, such as idazoxan (3), CH-39083 (12), or RW (9) inhibit TNF-α production after endotoxemia suggests that NE enhances TNF-α production via the stimulation of α2-adrenoceptors (presumably on Kupffer cells). Although it remains unknown whether infusion of NE alters α2-adrenoceptors, we have conducted a preliminary study to determine whether Kupffer cell α2-adrenoceptor maximum binding capacity (Bmax, i.e., the maximal receptor number) and dissociation constant (Kd) are altered during early sepsis. To determine this, the receptor binding assay was used as we (11, 44) previously described. Briefly, freshly isolated Kupffer cells (106) from sham and septic animals at 2 h after CLP were incubated with [3H]yohimbine (a radioactively labeled α2-adrenoceptor antagonist; sp act 79.2 Ci/mmol; DuPont NEN; final concentration, 2–64 nM in a volume of 200 μl) with or without 10 μM unlabeled yohimbine for 30 min at 37°C in an assay buffer (40 mM Tris-HCl and 10 mM MgCl2, pH 7.5) (28). Depending on the concentration of [3H]yohimbine, nonspecific binding was found to be 20–65% of total binding capacity. The values of Bmax and Kd were determined by Scatchard analysis (47). The results (average of 2 rats in each group) indicate that Bmax was 18.8 fmol/106 cells in sham animals and increased to 24.3 fmol/106 cells at 2 h after CLP (increased by 29%). In contrast, Kd decreased from 46.9 nM in sham animals to 17.3 nM at 2 h after CLP (decreased by 63%). Because Kd represents 1/affinity, the decreased Kd in septic animals suggests an increase in receptor affinity. Thus Kupffer cell α2-adrenoceptor binding capacity and affinity increase during early sepsis. In contrast, Kupffer cell cAMP levels decreased from 7.31 ± 0.14 pmol/5 × 106 cells in sham-operated animals (n = 5) to 3.82 ± 1.70 pmol/5 × 106 cells at 2 h after CLP (n = 4; P = 0.05; decreased by 48%). Since stimulation of α2-adrenoceptors reduces adenylate cyclase activity via the inhibitory GTP-binding protein (23), the decreased Kupffer cell cAMP level during early sepsis appears to be due to the increased α2-adrenoceptor binding capacity and affinity. In view of the previous reports (10, 21) demonstrating that circulating levels of NE increase significantly during sepsis, we propose that the increased binding capacity of α2-adrenoceptors on Kupffer cells during sepsis despite the elevated levels of NE plays a critical role in upregulating TNF-α production. Thus blockade of Kupffer cell α2-adrenoceptors should reduce TNF-α release and prevent hepatocellular dysfunction. The present study indicates that in vivo and in vitro administration of RW protects hepatocellular function and attenuates TNF-α production during early sepsis as well as after NE administration. Thus administration of α2-adrenergic antagonists appears to be a novel approach for maintaining cellular functions during sepsis. It should be noted that portal infusion of the α2-adrenergic antagonist RW at the doses used in this study did not alter the cardiac output, MAP, or heart rate. However, although administration of RW in septic animals prevented the occurrence of hepatocellular dysfunction and upregulation of TNF-α, future studies are needed to determine whether the blockade of α2-adrenoceptors by RW in normal rats alters ICG clearance and TNF-α production.

Intraportal administration of NE was performed, because recent study (49) has clearly indicated that the gut is the major source of NE release during sepsis. However, it could be argued that infusion of NE via the portal vein may cause maldistribution of hepatic perfusion. Although some redistribution of hepatic blood flow might have occurred under such conditions, we (45) have recently conducted studies to produce systemic NE levels similar to those observed in sepsis by implantation of a peritoneal miniosmotic pump (consistently releasing NE). The results (45) indicate that sustained elevation of systemic levels of NE produces significant depression in hepatocellular function as evidenced by reduced Vmax and Km of ICG clearance. The findings that perfusion of the isolated livers with NE reduced ICG clearance but did not alter alanine aminotransferase (49) or bile production (Fig. 3) suggest that NE at the concentration used in the present study does not appear to cause significant maldistribution of hepatic flow. Moreover, in the early stage of sepsis when plasma levels of NE increased to ~20 nM (10), hepatocellular dysfunction occurred despite the fact that hepatocellular perfusion increased as demonstrated by various techniques such as radioactive microspheres, laser Doppler flowmetry, galactose clearance, and colloid carbon infusion (41). Thus it is unlikely that NE-induced hepatocellular dysfunction is mainly due to significant maldistribution of hepatic blood flow by this catecholamine.
Because NE and RW may affect hepatic perfusion, it could be argued that the change of hepatic blood flow may have altered in vivo ICG clearance in the present study. However, this does not appear to be the case. Paumgartner et al. (27) suggested that the capacity of liver to remove ICG has a maximal limit. Their studies (27) also indicate that the classic Michaelis-Menten kinetics (with Lineweaver-Burk plot) could be applied to the initial ICG uptake in the rat and human livers. Paumgartner et al. (27) also postulated that when all hepatocyte receptor/carrier sites for ICG are occupied, removal capacity is at its maximum. Because saturation can theoretically be obtained despite fluctuations in hepatic blood flow and other variables and it is not possible to determine ICG clearance at an extremely high dose of this agent, maximal velocity of ICG clearance can be determined from three or more submaximal doses of ICG. This appears to be an ideal method for evaluating hepatocellular function independent of changes in hepatic perfusion (43). It should be pointed out that hepatocellular dysfunction observed during early stages of sepsis (i.e., 2–10 h after CLP) does not appear to be due to any reduction in hepatic blood flow. This conclusion is based on the findings that hepatic perfusion increases at 2–10 h after CLP and V_max and K_m of ICG clearance decrease at the same time points after the onset of sepsis (41). Thus hepatocellular dysfunction is not a flow-related event during early sepsis. In addition, since hyperdynamic circulation occurs at 2 h and hepatocellular function is depressed at 1.5 h after CLP, it appears that hepatocellular dysfunction, observed in the very early stage of sepsis, is not due to hyperdynamic circulation or hypermetabolism-related events (36).

Although the precise signal transduction mechanism responsible for the activation of α2-adrenoceptors on macrophages or Kupffer cells remains unknown (28), α2-adrenoceptors are associated with the inhibition of adenylate cyclase via the inhibitory GTP-binding protein subunit and subsequent suppression of intracellular cAMP (4). It is thought that stimulation of α2-adrenoceptors enhances TNF-α production through the reduction of intracellular cAMP levels (13). In this regard, our preliminary data have indicated that intracellular cAMP level in Kupffer cells decreases by 48% at 2 h after the onset of sepsis. A second mechanism by which TNF-α may be upregulated by NE is α2-adrenoceptor-mediated calcium flux into Kupffer cells, resulting in increased intracellular calcium levels (4). Studies (22, 46) have shown that an increase in macrophage calcium plays an important role in upregulating TNF-α production. Therefore, maintenance of intracellular cAMP levels and/or blockade of calcium influx appears to be an effective approach for attenuating the increased release of this inflammatory cytokine from Kupffer cells due to NE stimulation. However, it remains to be determined whether the beneficial effects of RW on TNF-α and hepatocellular function observed in the current study are indeed due to changes in Kupffer cell cAMP levels and/or calcium flux during early sepsis.

In summary, our results indicate that administration of the α2-adrenergic agonist NE in normal animals depressed hepatocellular function. Coadministration of NE with the specific α2-adrenergic antagonist RW, however, prevented the occurrence of NE-induced hepatocellular dysfunction. Moreover, intravenous administration of RW early after the onset of sepsis prevented sepsis-induced hepatocellular dysfunction and downregulated production of TNF-α. These results, taken together, suggest that gut-derived NE-induced hepatocellular dysfunction in early sepsis is mediated by the activation of α2-adrenoceptors, which appears to be responsible for stimulating Kupffer cells to enhance the release of TNF-α. Thus modulation of hepatic responsiveness to NE by α2-adrenergic antagonists should provide a novel approach for maintaining cell and organ functions during sepsis.

This study was supported by National Institute of General Medical Sciences Grant GM-53008 (P. Wang). P. Wang is the recipient of National Institutes of Health Independent Scientist Award K02-AI-01461.

REFERENCES


