Prevention of Kupffer cell-induced oxidant injury in rat liver by atrial natriuretic peptide

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Bilzer, Manfred, Hartmut J aeschke, Angelika M. Vollmar, Gustav Paumgartner, and Alexander L. Gerbes. Prevention of Kupffer cell-induced oxidant injury in rat liver by atrial natriuretic peptide. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1137–G1144, 1999.—The generation of reactive oxygen species (ROS) by activated Kupffer cells contributes to liver injury following liver preservation, shock, or endotoxemia. Pharmacological interventions to protect liver cells against this inflammatory response of Kupffer cells have not yet been established. Atrial natriuretic peptide (ANP) protects the liver against ischemia-reperfusion injury, suggesting a possible modulation of Kupffer cell-mediated cytotoxicity. Therefore, we investigated the mechanism of cytoprotection by ANP during Kupffer cell activation in perfused rat livers of male Sprague-Dawley rats. Activation of Kupffer cells by zymosan (150 µg/ml) resulted in considerable cell damage, as assessed by the sinusoidal release of lactate dehydrogenase and purine nucleoside phosphorylase. Cell damage was almost completely prevented by superoxide dismutase (50 U/ml) and catalase (150 U/ml), indicating ROS-related liver injury. ANP (200 nM) reduced Kupffer cell-induced injury via the guanylyl cyclase-coupled A receptor (GCA receptor) and cGMP; mRNA expression of the GCA receptor was found in hepatocytes, endothelial cells, and Kupffer cells, and the cGMP analog 8-bromo-cGMP (8-BrcGMP; 50 µM) was as potent as ANP in protecting from zymosan-induced cell damage. ANP and 8-BrcGMP significantly attenuated the prolonged increase of hepatic vascular resistance when Kupffer cell activation occurred. Furthermore, both compounds reduced oxidative cell damage following infusion of H2O2 (500 µM). In contrast, superoxide anion formation of isolated Kupffer cells was not affected by ANP and only moderately reduced by 8-BrcGMP. In conclusion, ANP protects the liver against Kupffer cell-related oxidant stress. This hormonal protection is mediated via the GCA receptor and cGMP, suggesting that the cGMP receptor plays a critical role in controlling oxidative cell damage. Thus ANP signaling should be considered as a new pharmacological target for protecting liver cells against the inflammatory response of activated Kupffer cells without eliminating the vital host defense function of these cells.

guanosine 3′,5′-cyclic monophosphate; cytoprotection; liver injury; liver perfusion; reactive oxygen species

ISCHEMIA-REPERFUSION INJURY is an important determinant for the success of liver transplantation. This injury contributes to primary nonfunction, dysfunction, and nonanastomotic biliary stenosis and is therefore pivotal for morbidity and mortality after liver transplantation (26). Thus better protection against ischemia-reperfusion injury is urgently needed.

Several pathomechanisms contribute to ischemia-reperfusion injury of the liver. Lack of oxygen during ischemia induces depletion of ATP, followed by a deterioration of intracellular Ca2+ and Na+ homeostasis (9, 18) and the activation of cytotoxic enzymes such as proteases (17). Additional damage occurs during reperfusion. Reactive oxygen species (ROS) have been implicated in the pathogenesis of hepatic reperfusion injury (15, 20). Several studies revealed that activated Kupffer cells contribute to posts ischemic oxidant stress during initial reperfusion (8, 21). Furthermore, the activation of Kupffer cells induces a complex network of cytokines, which participates in sinusoidal accumulation of granulocytes and microcirculatory failure (10, 12, 23). Kupffer cell activation and the subsequent vascular inflammation also play a central role in liver injury induced by endotoxin (23, 32). This potent activator of Kupffer cells translocates across the gut into donor organs and causes graft failure after liver transplantation (35, 40). Thus pharmacological interventions directed toward a reduction of Kupffer cell-related cell damage may protect the liver against reperfusion injury, however, these interventions have not yet been established.

Recently, we have shown that atrial natriuretic peptide (ANP) protects the liver against ischemia-reperfusion injury (6, 19). This circulating hormone released by the heart in response to volume expansion also preserves kidney function after renal ischemia and reperfusion (34, 37). The mechanisms of ANP-mediated cytoprotection remain to be elucidated and may provide insight into signal transduction processes as possible targets of pharmacological intervention. ANP has received attention mainly for its vasodilating properties (4, 27). However, recent evidence shows that ANP has an impact on other biological functions, e.g., on the immune system (43, 44). In this respect, an influence of ANP on macrophage activation has recently been observed (24). On the basis of these findings, a possible modulation of Kupfer cell s, the resident macrophages of the liver, by ANP could be hypothesized. Alternatively, ANP might counteract consequences of Kupffer cell activation, such as liver cell damage by ROS. Thus potential hazards of other protective approaches interfering with the inflammatory response, such as suppression of the Kupffer cell-related host defense, would be avoided.
Two major subtypes of ANP receptors have been characterized and are present in the liver (33, 41). The ANP A receptor is coupled to particulate guanylyl cyclase (the GCA receptor) and thus is responsible for cGMP-mediated ANP effects, in particular vasodilation and decreased cytosolic free Ca\(^{2+}\) (27). The ANP C receptor functions predominantly as a clearance receptor and lacks guanylyl cyclase activity but may decrease adenylyl cyclase activity (27). The GCA receptor has been demonstrated in isolated hepatocytes (46), but, until now, it remains unclear whether this receptor is also expressed in nonparenchymal liver cells.

Therefore, the aim of our investigation was to study the protective effects of ANP on Kupffer cell-derived injury in the perfused rat liver. This model allows the selective activation of Kupffer cells by zymosan (cell wall particles from yeast) (14) without concomitant effects from neutrophils and extrahepatic macrophages. The sinusoidal release of lactate dehydrogenase (LDH) and purine nucleoside phosphorylase (PNP) were determined as parameters of cell damage. To characterize the mechanisms of ANP action, we investigated expression of the GCA receptor in parenchymal and nonparenchymal liver cells and the effects of its second messenger cGMP. Furthermore, direct ANP effects on reactive oxygen formation were investigated in isolated Kupffer cells.

**MATERIALS AND METHODS**

Animals and materials. Male Sprague-Dawley rats were purchased from Sawo (Kieslegg, Germany) and housed in a temperature- and humidity-controlled room under a constant 12:12-h light-dark cycle. The animals had free access to water and rat chow (standard diet, Altromin 1314, Lage, Germany). All experiments were performed with nonfasting rats weighing 250–300 g. The animals received humane care in compliance with guidelines of the local animal welfare committee. ANP was obtained from Novabiochem (Bad Soden, Switzerland). Zymosan, superoxide dismutase (SOD), H\(_2\)O\(_2\), inosine, xanthine oxidase, 8-bromo-cGMP (8-BrcGMP), cytochrome c, and Salmonella enteritidis endotoxin were purchased from Sigma Chemical (St. Louis, MO). Catalase was obtained from Boehringer (Mannheim, Germany). Oligonucleotides were purchased from MWG-Biotech (Ebersberg, Germany), and avian myeloblastosis virus RT was from Promega (Heidelberg, Germany).

Isolated perfused rat liver. Rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). After incision of the abdominal wall, the portal vein was cannulated with a 14-gauge Teflon intravenous catheter and the liver was perfused at a constant flow rate of 3.5 ml·min\(^{-1}\)·g liver\(^{-1}\) with Krebs-Henseleit buffer (38). The inferior vena cava was then cannulated via the right atrium and ligated above the right renal vein. After the bile duct was cannulated with PE-10 tubing, the liver was dissected free and transferred to a perfusion chamber.

In all experimental groups, livers were perfused with Krebs-Henseleit buffer at 37°C in a nonrecirculating fashion. The Krebs-Henseleit buffer contained (in mM) 118 NaCl, 4.8 KCl, 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 7 H\(_2\)O\(_2\), 1.5 CaCl\(_2\), and 25 NaHCO\(_3\). The perfusion buffer was gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\) by an oxygenator as described earlier (38). The temperature was continuously monitored with a thermistor probe inserted between the lobes of the liver. Bile flow was measured gravimetrically in prepared tubes. Perfusion pressure was monitored manometrically from tubing attached to the inflow cannula.

**Experimental protocol.** Eight groups of rat livers were studied. Groups 1–4 were subjected to zymosan (150 µg/ml) as follows. For group 1, zymosan was administered from minute 40 to 46 after perfusion was started (n = 5); for group 2, ANP (2, 20, or 200 nM) was administered from minute 30 to 50 and zymosan from minute 40 to 46 (n = 4); for group 3, SOD (50 U/ml) and catalase (150 µU/ml) were administered from minute 30 to 50 and zymosan from minute 40 to 46 (n = 4); and for group 4, 8-BrcGMP (50 µM) was administered from minute 30 to 50 and zymosan from minute 40 to 46 (n = 4).

Groups 5–8 underwent perfusion with H\(_2\)O\(_2\) as follows. Group 5 rats were continuously perfused with Krebs-Henseleit buffer for 100 min (n = 6); for group 6, H\(_2\)O\(_2\) (500 µM) was administered from minute 30 to 45 after perfusion was started (n = 5); for group 7, ANP (200 nM) was given from minute 10 to 50 of perfusion and H\(_2\)O\(_2\) (500 µM) from minute 30 to 45 (n = 4); and for group 8, 8-BrcGMP (50 µM) was administered from minute 10 to 50 and H\(_2\)O\(_2\) (500 µM) from minute 30 to 45 (n = 4).

Rat ANP, 8-BrcGMP, SOD, catalase, and H\(_2\)O\(_2\) were dissolved in sodium chloride (0.9%). Stock solutions were infused into the portal inflow of the perfusion system by microinfusion pumps. Zymosan suspensions were kept at 95°C for 30 min to destroy endogenous phospholipase A\(_2\) activity (14). Zymosan suspensions were then diluted in Krebs-Henseleit buffer, yielding a final concentration of 150 µg/ml.

Isolation of parenchymal and nonparenchymal liver cells. Cells were isolated from rats treated for 3 h with 2 mg/kg Salmonella enteritidis endotoxin or 1 ml/kg saline. Hepatocytes were isolated after collagenase digestion, and hepatic endothelial cells and Kupffer cells were separated by centrifugal elutriation as described in detail (22). Each cell fraction was repeatedly washed with Hanks’ balanced salt solution (HBSS) and was >95% pure as assessed by morphology, peroxidase staining, and superoxide formation. Cell viability for each fraction was >90% for hepatocytes and >95% for Kupffer cells and endothelial cells as determined by trypan blue exclusion. Cells were used for either superoxide measurement (Kupffer cells) or total RNA isolation using the guanidinium thiocyanate method as described in detail (16).

Expression of natriuretic peptide receptors. mRNA was isolated by adsorption of total RNA to oligo(dT) magnetic beads (Promega) and quantified by ultraviolet adsorption. mRNA was reverse transcribed with avian myeloblastosis virus RT and used for PCR (24, 41). Amplification with a primer specific for the GCA receptor was performed as described elsewhere (42). Oligonucleotides used for PCR amplification had the following sequences: GCA receptor gene antisense primer, 5'-AAGCTGATAATCCTGAGTACT-3' and sense primer, 5'-TTGCCAGCTGGTCTCCATTGTCA-3' (42).

Amplification products were separated by agarose gel electrophoresis and stained with ethidium bromide. As control for possible contaminations, mRNA instead of CDNA was amplified in parallel and no DNA bands were visible at electrophoresis.

Analytic methods. Sinusoidal efflux rates of LDH and PNP were measured as indexes of liver cell damage (36, 38). The activity of LDH in the perfusate was analyzed according to a standard test (2). PNP activity was determined by the xanthine oxidase-coupled formation of uric acid at 293 nM (36).

Superoxide anion formation of isolated Kupffer cells was measured by ferricytochrome c reduction (22). Approximately
RESULTS

Kupffer cell-induced liver injury. Kupffer cells of isolated perfused rat livers were activated by a 6-min infusion of zymosan (150 µg/ml), which is taken up by Kupffer cells but not by hepatocytes or endothelial cells (14). Liver injury was assessed from sinusoidal efflux of LDH and PNP. During zymosan infusion, LDH and PNP efflux increased only slightly and then returned to basal values. Forty minutes after zymosan infusion started, LDH and PNP efflux markedly increased (Fig. 3). In contrast, untreated livers showed LDH and PNP efflux rates of only 4 ± 2 and 2 ± 0.1 mU·min⁻¹·g⁻¹ liver⁻¹, respectively, after 100 min of perfusion. Thus considerable cell damage was induced by activation of Kupffer cells.

When Kupffer cells were activated in the presence of SOD (50 U/ml) and catalase (150 U/ml), sinusoidal LDH and PNP release increased only 12 ± 10 and 1.3 ± 0.5 mU·min⁻¹·g⁻¹ liver⁻¹ (P < 0.05) (Fig. 1). Because SOD and catalase selectively detoxify O₂⁻ and H₂O₂, these ROS or the subsequent products may contribute to cell damage after Kupffer cell activation.

Pretreatment of the livers with 200 nM ANP resulted in a similar attenuation of zymosan-induced efflux of LDH and PNP to 15 ± 6 and 2.1 ± 0.9 mU·min⁻¹·g⁻¹ liver⁻¹, respectively, at 100 min (P < 0.05) (Fig. 2). Pretreatment with 20 nM ANP showed similar influences on LDH efflux (8 ± 1 mU·min⁻¹·g liver⁻¹), whereas PNP efflux was unaffected (6.4 ± 3.4 mU·min⁻¹·g liver⁻¹) (Fig. 2). In the presence of 2 nM ANP, neither LDH efflux (52 ± 19 mU·min⁻¹·g liver⁻¹) nor PNP efflux (5.5 ± 2.9 mU·min⁻¹·g liver⁻¹) was influenced.

Similar protective effects were observed in livers pretreated with the cGMP analog 8-Br-cGMP (Fig. 3). After zymosan infusion, LDH and PNP release increased to only 13 ± 9 and 1.2 ± 0.4 mU·min⁻¹·g liver⁻¹. Therefore, protective effects of ANP seem to be mediated by the GCA receptor.

Hemodynamic effects of Kupffer cell activation. During zymosan infusion, portal pressure increased transiently from 4.0 ± 0.2 to 18.8 ± 2.8 cmH₂O. When zymosan administration was terminated, portal pressure declined rapidly but remained elevated above baseline levels (6.6 ± 0.7 cmH₂O, P < 0.05) (Fig. 4). These results indicate a prolonged deterioration of the hepatic circulation after Kupffer cell activation. Initial increase of portal pressure was not affected by pretreatment with 200 nM ANP, but, during further perfusion, portal pressure returned to baseline (4.0 ± 0.4 cmH₂O) (Fig. 4). Similar influences on the zymosan-induced increase of portal pressure were observed with 20 nM ANP (4.6 ± 1.1 cmH₂O). In contrast, 2 nM ANP showed no hemodynamic effects. The hemodynamic effects of 20 and 200 nM ANP were mimicked by 8-Br-cGMP (50 µM) (Fig. 4), suggesting a GCA receptor-cGMP-mediated attenuation of the prolonged increase of vascular resistance induced by activated Kupffer cells.

GCA receptor expression in parenchymal and non-parenchymal liver cells. Hepatocytes, Kupffer cells, and endothelial cells were isolated from untreated livers by enzymatic digestion and centrifugal elutriation. After

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1.0 × 10⁶ cells in HBSS containing 50 µM ferricytochrome c were plated on six-well culture plates in the absence or presence of SOD (500 U/ml). After a preincubation period of 20 min with or without 200 nM ANP or 50 µM 8-Br-cGMP, Kupffer cells were activated by opsonized zymosan (150 µg/ml). The cell suspension was then incubated at 37°C for 60 min in a humidified environment containing 5% CO₂. The amount of SOD-inhibitable ferricytochrome c reduction was measured with a molecular extinction coefficient of 21.1 mmol·l⁻¹·cm⁻¹ from the difference in absorbance at 550 nm (22).

Statistics. All data are expressed as means ± SD. Statistical significance between the control group and a treated group was determined with the paired or unpaired Student’s t-test. Comparisons between multiple groups were performed with one-way ANOVA followed by Bonferroni t-test. P < 0.05 was considered significant.

Fig. 1. Effect of Kupffer cell activation on sinusoidal lactate dehydrogenase (LDH) and purine nucleoside phosphorylase (PNP) release. Kupffer cells of perfused rat livers were activated by infusion of zymosan (150 µg/ml) for 6 min (●, n = 6). Compared with untreated livers (○, n = 6), LDH and PNP efflux rates significantly increased when Kupffer cells were activated. Administration of superoxide dismutase (SOD; 50 U/ml) and catalase (CAT; 150 U/ml) resulted in a significant attenuation of LDH and PNP efflux (▲, n = 4). Error bars indicate ± SD. **P < 0.05 compared with untreated livers; * P < 0.05 compared with zymosan-treated livers.
reverse transcription of mRNA, 200 ng of cDNA were amplified with a specific primer of the GCA receptor. Specific transcripts of the GCA receptor were found in hepatocytes, Kupffer cells, and endothelial cells (Fig. 5). These findings are in accordance with the earlier described particulate guanylyl cyclase activity in hepatocytes and moreover show for the first time expression of the GCA receptor in nonparenchymal liver cells.

Effect of ANP on \( \text{O}_2^\bullet^- \) generation of isolated Kupffer cells. The marked attenuation of Kupffer cell-induced liver injury by ANP suggests a reduction of ROS production or of ROS-related cell damage by activated Kupffer cells. To test this hypothesis, the influence of ANP on \( \text{O}_2^\bullet^- \) generation of isolated Kupffer cells was investigated. In agreement with earlier studies by Bautista et al. (1), basal \( \text{O}_2^\bullet^- \) production of Kupffer cells from lipopolysaccharide-treated rats was 1.37 ± 0.25 and increased to 3.58 ± 0.56 nmol·h\(^{-1}\)·10\(^6\) cells\(^{-1}\) (\( P < 0.05 \)) after administration of opsonized zymosan (final concentration of 150 \( \mu \)g/ml). Preincubation of Kupffer cells with 200 nM ANP for 20 min reduced basal and
zymosan-stimulated O$_2^-$ release by 15 and 18%, respectively, which was not significant ($P > 0.05$). Treatment with 50 μM 8-BrcGMP resulted in a moderate attenuation of O$_2^-$ formations by 28 and 36%, respectively ($P < 0.05$).

Effect of ANP on H$_2$O$_2$-induced liver damage. The lack of effect of ANP and the only moderate reduction of O$_2^-$ release of isolated Kupffer cells by 8-BrcGMP suggest ANP-mediated mechanisms of protection against consequences of increased ROS formation. This hypothesis was tested by infusion of 500 μM H$_2$O$_2$ for 15 min. During H$_2$O$_2$ infusion, sinusoidal efflux of LDH and PNP increased to a maximum of 559 ± 151 and 8.3 ± 1.1 mU·min$^{-1}$·g liver$^{-1}$, respectively. ANP significantly reduced the H$_2$O$_2$-induced efflux of LDH to 272 ± 46 and of PNP to 3.2 ± 1.2 mU·min$^{-1}$·g liver$^{-1}$ (Fig. 6). Similar but gradually smaller influences on H$_2$O$_2$-induced cell damage were observed with 50 μM 8-BrcGMP (Fig. 6).

**DISCUSSION**

Recently, protection of the kidney as well as the liver against ischemia-reperfusion damage by ANP has been reported (6, 19, 34, 37). Recent data show an effect of ANP on macrophage activation (24). Because activation of Kupffer cells plays a key role in reperfusion injury of the liver (8, 21), modulation of Kupffer cell-related cell damage might be an additional mechanism of ANP-mediated hepatoprotection. We therefore studied the effect of ANP on Kupffer cell-derived injury of the perfused rat liver. This model allows the selective activation of Kupffer cells by phagocytosable particles such as zymosan (14) without concomitant effects from activated neutrophils and extrahepatic macrophages. Using this approach, we propose a novel concept of hepatoprotection through ANP signaling via the GCA receptor: protection of liver cells against Kupffer cell-induced injury without elimination of the vital function of Kupffer cells in host defense.

Liver injury by activated Kupffer cells. Activation of Kupffer cells in vivo induces a complex network of cytokines accompanied by vascular inflammation (10, 12, 23). Furthermore, there is evidence for a pivotal role of ROS in the complex relation of these pathophysiological events. Several studies revealed a relevant vascular oxidant stress by activated Kupffer cells, which contributes to reperfusion injury (8, 15, 20, 21). Our experiments support this hypothesis. The selective activation of Kupffer cells in the perfused rat liver by zymosan resulted in a severalfold increase of sinusoidal LDH and PNP efflux, indicating considerable cell damage. Treatment with SOD and catalase reduced the zymosan-stimulated efflux of LDH and PNP by ~70%. Because SOD and catalase selectively detoxify O$_2^-$ and H$_2$O$_2$, their protective effects indicate ROS-mediated liver cell damage by activated Kupffer cells. Recently, it has been shown that ROS may induce microcirculatory failure due to sinusoidal leukostasis (25, 30). Because livers were perfused with a leukocyte-free buffer in our experiments, other mechanisms of ROS-mediated liver cell damage must be proposed. These imply the initiation of a cascade of reactions by O$_2^-$ and H$_2$O$_2$, which induce lipid peroxidation and signal transduction via redox-sensitive transcription factors such as nuclear transcription factor-$\kappa$B (16, 20).

Protective effects of ANP. Treatment of livers with ANP reduced cellular damage, as indicated by a decrease of the sinusoidal efflux of LDH and PNP. The concentration of ANP was important for the protection: 200 nM ANP significantly reduced both LDH and PNP effluxes to reperfusion injury (8, 15, 20, 21). Our experiments support this hypothesis. The selective activation of Kupffer cells in the perfused rat liver by zymosan resulted in a severalfold increase of sinusoidal LDH and PNP efflux, indicating considerable cell damage. Treatment with SOD and catalase reduced the zymosan-stimulated efflux of LDH and PNP by ~70%. Because SOD and catalase selectively detoxify O$_2^-$ and H$_2$O$_2$, their protective effects indicate ROS-mediated liver cell damage by activated Kupffer cells. Recently, it has been shown that ROS may induce microcirculatory failure due to sinusoidal leukostasis (25, 30). Because livers were perfused with a leukocyte-free buffer in our experiments, other mechanisms of ROS-mediated liver cell damage must be proposed. These imply the initiation of a cascade of reactions by O$_2^-$ and H$_2$O$_2$, which induce lipid peroxidation and signal transduction via redox-sensitive transcription factors such as nuclear transcription factor-$\kappa$B (16, 20).

**Fig. 5.** Expression of the guanylyl cyclase-coupled A (GCA) receptor in parenchymal and nonparenchymal liver cells. cDNA (200 ng) was amplified by PCR. Amplification products were separated by agarose gel electrophoresis and stained with ethidium bromide. Specific transcripts for the GCA receptor (451 bp) were found in hepatocytes, endothelial cells, and Kupffer cells of untreated livers.

**Fig. 6.** Influence of ANP or 8-BrcGMP on H$_2$O$_2$-induced liver injury. Infusion of 500 μM H$_2$O$_2$ resulted in a marked increase of sinusoidal LDH and PNP efflux ($\bullet$, $n = 5$). Infusion of 200 nM ANP from 10 to 50 min ($\Delta$, $n = 5$) significantly attenuated LDH and PNP efflux rates (*$P < 0.05$). Minor effects were observed in 8-BrcGMP-treated livers ($\circ$, $n = 4$).
ANP protects against Kupffer cell-induced liver damage

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As discussed above, ROS release into sinusoids seems to be the major mechanism of cell damage after Kupffer cell activation in the perfused rat liver. Therefore, questions arise whether ANP modulates ROS production or their consequences. Recently, it has been shown that ANP and cGMP analogs can inhibit nitric oxide production of macrophages (24). This may reduce the production of highly toxic peroxynitrite, which is formed by the reaction of O$_2^-$ with nitric oxide. However, inhibition of nitric oxide synthetases by nitro-L-arginine methyl ester did not affect zymosan-induced cell damage in the perfused rat liver (5). Therefore, the hypothesis that ANP prevents ROS-related cell damage due to an attenuation of peroxynitrite formation seems unlikely.

A dose of 200 nM ANP did not affect O$_2^-$ production of isolated Kupffer cells following activation by zymosan. This lack of effect could be because of an impairment of ANP receptors during isolation of Kupffer cells with Pronase. The moderate attenuation of O$_2^-$ release by 8-BrcGMP is in accordance with this contention. This minor effect could contribute to prevention of Kupffer cell-related liver injury by ANP and 8-BrcGMP.

On the other hand, ANP reduced liver injury by an externally added oxidant. Because O$_2^-$ is a weak oxidant that rapidly dismutates to H$_2$O$_2$, oxidative liver injury was induced by the infusion of H$_2$O$_2$. Treatment of the liver with 200 nM ANP reduced cell damage by H$_2$O$_2$ as indicated by a significant decrease in sinusoidal LDH and PNP efflux. Similar effects were observed with 8-BrcGMP, suggesting protection through signaling by the GCA receptor and cGMP. These results clearly demonstrate that ANP protects liver cells against the oxidant stress from activated Kupffer cells. Our data suggest that inflammatory liver injury can be modulated by hormones such as ANP acting on the GCA receptor. Thus ANP signaling could be a newly recognized endogenous defense mechanism to protect the liver against cytotoxic products generated during host defense reactions or general activation of Kupffer cells, e.g., during ischemia-reperfusion (4). Therapeutically, this pathway could provide promising targets for interventions that selectively enhance the resistance of liver cells against inflammatory Kupffer cell toxicity without eliminating this vital host defense mechanism (31).

The molecular mechanisms of protection by ANP still remain to be elucidated. Stimulation of the GCA receptor results in the increased formation of cGMP, which interacts with three types of intracellular receptor proteins: cGMP-dependent protein kinases, cGMP-regulated cyclic nucleotide phosphodiesterases, and cGMP-regulated ion channels (28). Thus cGMP can alter cell function through protein phosphorylation or influences on the ion homeostasis, which depends strongly on the variable expression of cGMP receptor proteins in different cell types (28). In our experiments, protective ANP effects were mimicked by 8-BrcGMP. This cGMP analog binds with little or no affinity to the cGMP binding site of cGMP-regulated phosphodiesterases and is thus unable to activate cAMP hydrolysis.
(39). In contrast, 8-BrcGMP binds to cGMP-dependent protein kinases, which are considered as important regulators of cytosolic Ca\(^{2+}\) (11). As shown previously in isolated hepatocytes, ANP attenuates the increase in cytosolic Ca\(^{2+}\) following oxidant stress (45). Although the precise cGMP-dependent cellular alterations remain to be defined, the present study provides strong support for a crucial role of cGMP receptor proteins in regulating cellular protection against oxidant stress in the liver.

In conclusion, we demonstrated that ANP can protect liver cells against oxidant stress of activated Kupffer cells. Because ANP had no direct effect on superoxide formation of Kupffer cells and also protected against externally added hydrogen peroxide, we conclude that ANP enhances the resistance of target cells in the liver against ROS-mediated cytotoxicity. All ANP effects were mimicked by 8-BrcGMP, which indicates GCA receptor/cGMP signaling of cytoprotection. Therefore, cGMP receptor proteins seem to play a crucial role in regulating the extent of liver cell damage following oxidant stress. Thus ANP signaling appears to be an endogenous defense system to strengthen the resistance of cells against cytotoxic products of Kupffer cells. Therefore, ANP and its receptor and signaling pathway may be new promising therapeutic targets to protect liver cells against inflammatory cell injury.

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