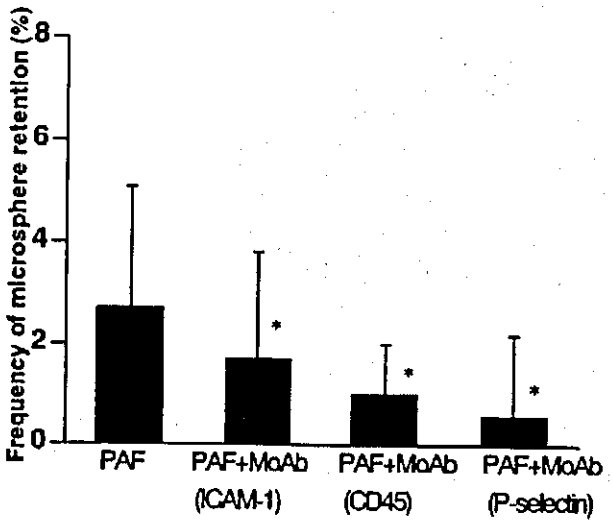

Fig. 6. Frequency of PFP-containing microsphere retention in mesenteric venules (each group, $n=21-30$ vessels) of Wistar rats (each group, $n=6$) under local application of PAF 10^{-8} M. Depletion of leukocytes with an antibody to CD45 or blockade of leukocyte attachment to postcapillary endothelium with a monoclonal antibody to ICAM-1 or P-selectin decreased microsphere retention to venular endothelium.

(Fig. 6). After PAF application following four PFP microsphere injections in all 60 observation fields, only two PFP microspheres entered the extravascular space from postcapillary venules which exhibited localized hemorrhage and enhanced leukocyte migration.

4. Discussion

The present study serves to demonstrate that the kinetics of most PFP microspheres were similar to those of erythrocytes in the microcirculation after intravenous administration. The microsphere retention is slightly but significantly enhanced in PAF-stimulated venules especially in regions with leukocyte adhesion.

4.1. Behavior of the microspheres in normal microcirculation

The significant correlation between the mean velocities of PFP microspheres and those of erythrocytes in arterioles, capillaries and venules in the present study is consistent with the results using air-filled sonicated albumin microspheres [20]. We recently reported that the unique myocardial enhancement by dodecafluoropentane based QW7437 microspheres persisting beyond the left ventricular cavity is partly due to frequent microsphere retention in normal venules (about 10%) [3]. The PFP microspheres, however, were less frequently retained ($0.3 \pm 0.6\%$) in venules. After intravenous injection, microspheres that are significantly larger than the pulmonary capillary vessel diameters may not cross the pulmonary microcirculation [2,3]. Thus, no transient or permanent occlusion by labeled PFP microspheres was found in arterioles or capillaries within the mesentery microcirculation in the present study.

4.2. Microsphere retention in inflamed venules

Villanueva et al. have found in an in vitro perfusion model, that air-filled albumin microspheres adhere to experimentally inflamed human coronary arterial endothelial cells without leukocytes and platelets more than controls [21], and that coating the microsphere shell with polyethylene glycol to mask electrostatic and hydrophobic properties inhibits microsphere–endothelium adherence [22]. They suggest that ionic and hydrophobic forces play a role in albumin air-filled microsphere adhesion to activated coronary arterial endothelial cells [22]. Damaged glycocalyx on the endothelial cells under inflammatory conditions such ischemia and reperfusion enhances the persistence of sonicated albumin microspheres within the myocardial microcirculation [5]. The present observations indicate that coadherence to endothelial cells of PFP microspheres and PMN leukocytes. This suggests a role for adhesion molecules on activated PMN leukocytes as a mechanism for microsphere retention in inflamed venules. Present data were compatible to the previous report by Lindner et al. [6].

PAF has been shown to play an important role in inflammatory reactions in a variety of organs, including ischemia–reperfusion induced tissue injury [7,23]. During the local application of 10^{-8} M PAF, microsphere retention was increased in venules, but not in arterioles or capillaries. Retained microspheres behaved like leukocytes that rolled on or adhered to venular cells. The initial attachment of the microspheres to the activated leukocytes or endothelial cells in postcapillary venules is probably induced by a random displacement of microspheres and leukocytes produced by interaction with erythrocytes [24]. This random dispersion serves to displace leukocytes [24] and some of the microspheres from the vessel center towards the vascular endothelial lining with reduced velocity. Leukocytes adhered to venular endothelial cells, but not to capillary or arterial endothelial cells even after PAF application. After attachment or phagocytosis of a microsphere by an activated leukocyte, the microsphere–leukocyte complex rolled on or adhered to inflamed venular endothelial cells. This phenomenon can explain why retained microspheres behaved like leukocytes that rolled on or adhered to venular endothelial cells. In fact, 90% of retained PFP microspheres were attached to adhering leukocytes and all the rolling microspheres in venules were attached to leukocytes. Leukopenia caused by the anti-CD45 MoAb decreased microsphere retention as well as leukocyte adherence to venular endothelial cells, providing further evidence for a role of leukocytes in the microsphere retention process. Blockade of leukocyte attachment to postcapillary venular endothelial cells with a MoAb to ICAM-1 [17,25] and P-selectin [17,18,26,27] also decreased the number of retained micro-

spheres attached to adhering leukocytes in venules. The data support the hypothesis that adhesion molecules on activated endothelial cells and activated leukocytes play an important role in the overall process of microsphere retention. MAC-1 on the leukocytes has been suggested to play a role on binding of leukocytes with albumin microspheres [6].

4.3. Experimental limitations

Although the size of the microspheres in vivo is an important factor controlling their kinetics in the microcirculation, accurate measurements of microsphere size in vivo could only be obtained in this study from images of fluorescently labeled microspheres moving rapidly in microvessels within about 7 μm. Second, our microscopic examination did not include ultrasonography. Several investigators have indicated that the acoustic output from a diagnostic ultrasound transducer causes transient changes in microsphere size, an increase in microsphere destruction rate [28,29], and damage to capillary endothelial cells during contrast ultrasonography [30–32]. Finally, the results of the present study may apply only to specific ultrasound contrast agents composed of gas filled, pre-formed, albumin-shell microspheres and not to other contrast agents containing the same or similar fluorocarbons [3].

4.4. Clinical implications

The present in vivo and in vitro observations suggest that leukocyte–endothelium–microsphere adhesion in the inflamed microcirculation may facilitate contrast ultrasonography with which to evaluate inflammatory distribution as well as tissue perfusion. In addition, our data suggest that not only microsphere–endothelium interaction but also microsphere–leukocyte and leukocyte–endothelium interaction should be considered in the development of site-targeted contrast microspheres [33,34].

5. Conclusion

Most of PFP microspheres following intravenous injection pass through the microcirculation in a manner similar to that of erythrocytes. A small subgroup of PFP microspheres was retained in venules of the inflamed microcirculation in the regions with leukocyte adhesion. Interaction among microspheres and activated leukocytes and venular endothelial cells plays an important role in the process of microsphere retention.

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Hydrogen sulfide as an endogenous modulator of biliary bicarbonate excretion in the rat liver

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Abstract

Cystathionine-γ-lyase (CSE) is an enzyme catalyzing cystathionine and cysteine to yield cysteine and hydrogen sulfide (H_2S), respectively. This study aimed to examine if H_2S generated from the enzyme could serve as an endogenous regulator of hepatobiliary function. Gas chromatographic analyses indicated that, among rat organs herein examined, liver constituted one of the greatest component of H_2S generation in the body, ranging at 100 μmol/g tissue, being comparable to that in kidney and 1.5-fold greater than in brain, where roles of the gas in regulation of neurotransmission were reported previously. At least half of the gas amount in the liver appeared to be derived from CSE, since blockade of the enzyme by propagylglycine suppressed it by 50%. Immunohistochemistry revealed that CSE occurs not only in hepatocytes but also in bile duct. In livers in vivo as well as in those perfused ex vivo, treatment with the CSE inhibitor induced choleresis by stimulating the basal excretion of bicarbonate in bile samples. Transportal supplementation of NaHS at 30 μmol/L, but not that of N-acetylcysteine as a cysteine donor, abolished these changes elicited by the CSE inhibitor in the perfused liver. The changes elicited by the CSE blockade did not coincide with alterations in hepatic vascular resistance, showing little involvement of vasodilatory effects of the gas in these events, if any. These results first provided evidence that H_2S generated through CSE modulates biliary bicarbonate excretion and is thus a determinant of bile salt-independent bile formation in the rat liver.

Key Words: hydrogen sulfide, cystathionine γ-lyase, biliary epithelium, bicarbonate, liver

Introduction

Cysteine metabolism in the liver has been shown to contribute greatly to detoxification processes through multiple mechanisms. Following reduction and decarboxylation processes, this amino acid is converted to taurine, the compound used for conjugation of bile acids. Cysteine serves as a substrate for synthesis of glutathione through reactions of glutamate ligase and glutathione synthase, and is also used to generate sulfate through aspartate transferase and sulfite oxidase; these two compounds have well been shown to play an important role in detoxification of xenobiotics such as acetaminophen. Another important substance generated upon cysteine metabolism in vivo is hydrogen sulfide (H_2S). This gaseous compound has recently been shown to account for a signaling molecule in neural and vascular systems. It is produced mainly by two types of pyridoxal-5'phosphate-dependent enzymes responsible for metabolism of L-cysteine, cystathionine γ-lyase (CSE, EC 4.4.1.1) and cystathionine β-synthase (CBS, EC 4.2.1.22). In other words, while the primary role of the two enzymes is to constitute the transsulfuration pathway that provides cysteine through biotransformation of methionine derived from nutrition, both CSE and CBS are able to use cysteine as the substrate to generate H_2S. Several lines of evidence support a hypothesis that H_2S serves as a neurovascular regulator in vivo (7). In brain, the gas is synthesized by CBS and has been reported to account for neuroprotectant (9). On the other hand, CSE-derived H_2S was shown to relax vascular smooth muscle cells through its ability to increase the conductance of potassium channels (24); in this study, H_2S released from the enzyme blocked vasoconstriction of rat aortic rings elicited by glybenclamide, a blocker of the ATP-gated K^+ channel. Furthermore, the CSE activities have been reported to be altered under disease conditions; the activity in the liver is reduced in patients with liver cirrhosis and in those exposed to surgical insults or acquired immune deficiency syndrome (11, 13, 23). On the other hand, experimental models of vitamin B_6 deficiency or streptozotocin-induced diabetes revealed alterations in CSE in the liver under these disease conditions (8, 18). Until now, however, effects of such alterations in the activities on organ functions and roles of H_2S under these circumstances have not fully

been investigated yet.

This study was designed to first focus on differences in contribution of CSE to tissue H_2S generation; the data indicated that the liver constitutes one of the largest organ components for the gas generation in the body. Based on this result, we further attempted to examine if H_2S derived from the enzyme could play a role in regulation of hepatobiliary function. The current results first provided evidence that the liver utilizes this gaseous substance as a modulatory determinant of biliary bicarbonate excretion.

Materials and Methods

In vivo and ex vivo determination of bile constituents.

The experimental protocols herein described were approved by our institutional guidelines provided by the Animal Care Committee of Keio University School of Medicine. Male Wistar rats weighing 220-260 g (CLEA Japan, Tokyo) were allowed free access to laboratory chow and tap water, and were fasted for 24 hrs prior to experiments. As described elsewhere, rats were anesthetized with an intramuscular injection of pentobarbital sodium at 50 mg/kg, and their common bile ducts were cannulated to collect bile samples. Bile output was monitored in vivo according to our previous method (10). When necessary, livers of these rats were perfused ex vivo with the oxygenated Krebs-Henseleit buffer at a constant flow rate of 4 mL/min/g liver in a single-pass mode (16)(17)(20)(21). Bile samples collected through a cannulation were used to determine concentrations of total bile salts, phospholipids, pH values and bicarbonate (HCO_3^-) according to previous methods described elsewhere (10)(17)(22).

Experimental protocols.

Propagylglycine (PPG) was used as a potent inhibitor of CSE. PPG was dissolved in physiological saline as a vehicle and administered at a dose of 1.5 mg/kg body weight intraperitoneally at 4 hrs prior to the preparation for bile duct cannulation. Bile was collected every 10 min until the end of experiments according to our previous method (16)(17). In case of experiments using the ex-vivo perfused preparation, livers were excised from the PPG-treated rats and perfused with the Krebs-Henseleit buffer containing 100 μmol/L of PPG to avoid a possible reduction of the enzyme blockade due to elimination of the reagent from the system. To examine effects of the intraperitoneal injection of the CSE inhibitor on endogenous H_2S generation, we determined tissue contents of the gas in vivo. Livers were excised and snap-frozen at 4 hrs after the treatment with PPG or the vehicle, and the samples were minced with 4% tetracholic acid to remove proteins. Amounts of H_2S in the liver tissues were determined by gas chromatography according to previous methods described elsewhere (6)(7). Briefly, 4.5 ml of homogenates containing 100 mg of tissue and

10mM NaOH were filled with N_2 gas and placed in a gas tight vial. H_2S gas was released by adding 1 ml of 100 % trichloroacetic acid to the vial with a syringe and then incubated at 60 ^{0}C for 10 min. Then, gas was removed from the reaction vial by gas tight syringe and applied to a gas chromatograph equipped with FPD. In separate sets of experiments, bile output was monitored every 10 min after establishment of the bile duct cannulation, and concentrations and fluxes of bile constituents were compared between the control and PPG-treated groups. In order to examine if effects of PPG are attributable to a reduction of the reaction product of CSE such as H_2S, we examined effects of supplementation of NaHS, a soluble donor of the gas at desired concentrations, in the buffer for the ex-vivo perfusion system. As a control set of the experiments, we compared effects of the same concentrations of N-acetylcysteine (NAC), a cysteine donor. In experiments using isolated ex-vivo perfused livers, sodium taurocholate was added to the buffer at desired concentrations in a range between 0 and 30 μmol/L. Using data collected from these experiments, bile acid-independent fraction of bile output was determined by plotting bile output as a function of biliary output of bile salts in the samples: the value of the output at y-intersection (zero concentration of bile salts) was regarded as the bile acid-independent fraction (2).

Immunohistochemistry

Liver tissues also served as samples for immunohistochemistry. An anti-CSE antibody was prepared by immunization to chicken of the C-terminus peptide CYGGTNRYFRRVASE, the sequence of which is identical to that of the rat enzyme. The antibody was purified from the antiserum using affinity choromatography as described elsewhere (3). The specificity of the antibody was confirmed by Western blot analyses. For immunohistochemistry, rat livers were removed to prepare O.C.T. compound-embedded frozen sections (7 μm). The sections were immunostained with the anti-CSE antibody using the Vectastain ABC kit (Vector Laboratories), as previously described (4)(5). Semiserial sections were stained with the anti-CSE antibody or with the anti-rat keratin 19 monoclonal antibody (MAB1675, Chemicon, Temecula, CA) to examine colocalization of the enzyme with biliary epithelium and hepatocellular bile canaliculi, when necessary.

Statistical analyses

The statistical significance of data among different experimental groups was determined by one-way ANOVA and Fischer's multiple comparison test. $P < 0.05$ was considered significant.

Results

Liver constitutes the largest organ component for CSE-derived H_2S generation.

Figure 1 illustrates tissue contents of H_2S in different organs. The control liver treated with vehicle contained approximately 80 μmol/g tissue of the gas (Panel A). Livers from rats pretreated with 1.5 mg/kg PPG, an inhibitor of CSE, suppressed the constitutive levels of the gas by 50%. The dose of PPG used in this experiment appeared to be sufficient enough to block the enzyme, as indicated by dose responses of the H_2S contents as a function of doses of the inhibitor (Panel B). When the tissue gas contents were compared among different organs (Panel C), liver appeared to constitute the largest organ component for endogenous H_2S production; the level was comparable to that measured in the kidney and 1.5-fold greater than that in the brain. So far as judged by sensitivity to PPG, the gas generation in the liver and kidney depended largely on CSE, while that in other organs such as brain, lung and spleen seemed CSE-independent; the finding is consistent with previous observation in mice brain tissues where CBS constitutes a major source for the gas generation (1)(9).

CSE-derived H_2S is a determinant of the basal bile output and biliary HCO_3^- excretion.

Figure 2 demonstrated protein expression of CSE in the rat liver tissues. Western blot analyses indicated that the purified polyclonal antibody used in this study specifically recognized the enzyme at 40 kDa (Panel A). The immunohistochemistry using the same antibody revealed that the most intense reactivities were seen in periductual regions of portal triads, while walls of hepatic arterial walls and terminal portal veins displayed little reactivities, if any. In addition, a modest expression of CSE was notable in hepatocytes, indicating intralobular homogeneity in its expression (Panel B), while non-specific IgG did not stain the slice (Panel C). Panels D and E illustrates semiserial sections stained with the anti-CSE and anti-keratin 19 antibodies, respectively. As seen, cytokelatin-positive ductular structures connecting to bile canalicular networks near the portal triad exhibited notable CSE expression, while an artery adjacent to the portal vessel did not display evident

immunoreactivities. The staining disappeared when the anti-CSE antibody was absorbed by adding the antigen peptide (data not shown).

We determined effects of systemic administration of PPG on bile output and biliary constituents in vivo according to the identical protocol used in Figure 1. As shown in Table 1, the PPG administration significantly stimulated basal bile output by 15%. The biliary concentration of HCO_3^- was also significantly elevated in the PPG-treated group. Since PPG inhibits CSE and could not only reduce endogenous H_2S but also modify cysteine metabolism, it is necessary to examine direct effects of the exogenous H_2S administration on hepatobiliary function. However, such experiments were difficult, since the administration of NaHS, the H_2S-donating reagent, is known to change systemic blood pressure in vivo through its vasorelaxing action (25). We thus used livers perfused ex vivo with the taurocholate-free Krebs solution to prove roles of CSE-derived H_2S in the basal bile excretion.

As illustrated in Figure 3, the hepatic vascular resistance was comparable among four groups tested (e.g. vehicle, PPG, PPG+NaHS and PPG+NAC). Under these circumstances, the basal bile output was significantly elevated by 20% in perfused livers of the PPG-treated rats as compared with those treated with vehicle. This response was slightly greater than that observed in the experiments in vivo (Table1), presumably because the perfusion of the organ was carried out under cholate-free conditions, as discussed later in Results. The choleretic response elicited by the PPG treatment was repressed by co-perfusion of NaHS at 30 μmol/L, the concentration being comparable to the PPG-sensitive fraction of the gas generation. On the other hand, co-perfusion of the same concentration of N-acetylcysteine (NAC), a reagent entering cells to yield cysteine, did not alter the CSE-elicited choleretic response. Likewise aforementioned observations in vivo (Table 1), the PPG treatment significantly enhanced biliary HCO_3^- concentrations, and co-perfusion of 30 μmol/L NaHS completely attenuated the changes in the perfused rat livers. On the other hand, the NAC co-perfusion did not repress the PPG-induced elevation of the HCO_3^- concentration (Panel B of Figure 3).

Since HCO_3^- serves as a putative constituent yielding the driving force for bile

formation, we determined if the bile acid-independent bile formation is elevated in livers of the PPG-treated groups. As seen in Figure 5, where the output was plotted as a function of biliary fluxes of bile salts, Y-intersection of the line for the PPG-pretreated groups became markedly decreased and dissociated from that for the control groups. Difference between the two groups became smaller with increasing the fluxes of bile salts, but the difference was still evident when the flux of bile salts reached the physiologic levels (70 nmol/min/g liver). Such a dependency of the PPG effect on bile salts was consistent with the current data indicating differences in the choleretic responses between in-vivo (Table 1) and ex-vivo (Fig 3) perfused livers. We further investigated whether biliary output of glutathione, another major constituent for bile acid-independent bile formation, could also be elevated under the blockade of CSE. As seen in Figure 4, total amounts of glutathione excreted into bile was comparable among the three groups, suggesting that this constituent plays little role in generation of the osmotic driving force, if any. Interestingly, the ratio between reduced and oxidized forms of glutathione (GSH/GSSG) was significantly elevated by the CSE blockade with PPG. Moreover, the PPG-induced elevation of GSH/GSSG in bile was further elevated with co-perfusion with 30 mmol/L NaHS. As one might expect, the PPG pretreatment significantly caused a reduction of total glutathione presumably through inhibition of the transsulfuration pathway. The PPG-elicited decrease in hepatic glutathione contents was unchanged upon administration of NaHS, suggesting that the event is not mediated by endogenous H_2S. Among the three groups, greater than 90% of glutathione was present as the reduced form (data not shown). These results suggest that suppression of CSE-derived H_2S accelerates biliary excretion of GSH, while its hepatic contents are reduced. Moreover, exogenous supplementation of the gas under the CSE blockade further increases its excretion into bile. Physiologic implications of this phenomenon will be mentioned later in Discussion. Collectively, the present results suggest that H_2S endogenously generated by CSE modulates the basal excretion of HCO_3^- in bile, playing a role in regulation of the basal bile output through mechanisms dependent on bile acid-independent choleresis.

Discussion

The present study first provided evidence for the presence of considerable amounts of H_2S in the liver. Furthermore, the gas appeared to serve as an endogenous modulator of the basal bile formation in the liver. Mechanisms for regulation of the basal bile formation involve the bile acid-independent process rather than bile acid-dependent one. Several lines of the current data support this concept: First, the effect of blockade of CSE, the enzyme producing approximately 50% of the basal H_2S generation, causes an increase in the bile acid-independent bile output ex vivo and in vivo. Second, so far as judged from data from ex-vivo perfused livers, the difference in the excretion between PPG-treated and –untreated groups becomes increased as the biliary excretion of bile salts is reduced (in Fig 5), suggesting that the bile acid-independent fraction plays a major role. Thirdly and most importantly, between the two major biliary constituents for this fraction, HCO_3^- but not glutathione is elevated upon the CSE blockade and repressed by supplementation with H_2S, indicating that the former is attributable to generate the driving force for the bile formation. These results collectively suggest that stimulation of HCO_3^- plays an important role in the bile acid-independent choleresis elicited by suppression of CSE-derived H_2S generation.

As seen in alterations in hepatic and biliary contents of glutathione, PPG did not only suppress CSE-derived H_2S but also reduced the glutathione contents. Since the decrease in the hepatic glutathione contents was not restored by supplementation of NaHS, this event is not mediated by the gas but occurs as a consequence of blockade of CSE-dependent transsulfuration processes. Of interest is that biliary excretion of total glutathione [reduced (GSH) and oxidized (GSSG) forms of glutathione] was unchanged despites the reduction in their hepatic contents. Furthermore, the relative amounts of GSH in bile were increased with supplementation of NaHS. Considering biochemical properties of the gas as a potent reductant with small molecular weight, this result raised a possibility that exogenously administered H_2S is utilized to increase reducing equivalents for GSH in bile. Several processes should be taken into accounts for mechanisms by which exogenous H_2S increases the ratio of GSH/GSSG in bile: First, the blockade of CSE by PPG could

inhibit the conversion of cysteine into H_2S and thereby save this amino acid for the glutathione synthesis even when the supply of the substrate from transsulfuration pathway is inhibited. Such an event suggests roles of CSE-mediated conversion of cysteine into H_2S for a fail-safe mechanism to maintain the reducing equivalent. Secondly, exogenously administered H_2S could help reduction of cystine into cysteine and facilitates the entry of cystine from the extracellular space into cells, as first demonstrated in neurons by Kimura, et al (9). At the same time, H_2S has recently been shown to activate gamma-glutamylcysteine synthetase and secondarily upregulate GSH synthesis (9). Thus, roles of this gaseous mediator for regulation of glutathione metabolism in the liver deserves further studies provided for evidence that the gas serves as a redox-dependent regulator of entry and excretion of thiols in hepatocytes.

Among gaseous substances detected in mammalian tissues, H_2S has recently been suggested to account for a novel neurovascular transmitter, while receptor mechanisms for the gas signal transduction remain largely unknown. The current results first suggest that the liver could have the ability to execute remodeling of HCO_3^- excretion and increase the basal bile formation when exposing to disease conditions causing a decrease in the enzyme activity: such circumstances involves cirrhosis and surgical insults as previously reported both experimentally and clinically (11, 23). When considering effects of other gaseous mediators on a quality control of bile excretion, which were previously reported from our and other laboratories, it is not unreasonable to hypothesize that the liver could utilize multiple gases to regulate biliary function under physiologic and pathologic conditions. In the rat model of endotoxemia, NO suppresses oxidative phosphorylation via blockade of mitochondrial cytochrome *c* oxidase, and thereby down-regulates bile acid-dependent bile formation (20, 22). Although mechanisms for transcriptional regulation of the CSE expression remains largely unknown, previous studies revealed that exposure to excess NO caused up-regulation of the CSE expression in aortic tissues and increased endogenous generation of H_2S to modulate the vascular tone. As shown in the current study, the excess dose of exogenous NaHS supplementation reduced the basal bile output, suggesting that H_2S causes cholestasis with its excess amounts. In this context, quantitative determination of

these two gases in the endotoxemic liver deserves further studies provided that the functional link of their overproduction to biliary function could be demonstrated.

On the other hands, the current results together with our previous data collectively suggest that a reduction of H_2S and an increase in CO share common roles in regulation of bile formation in that both events stimulate excretion of bile constituents besides bile salts. CO at micromolar levels not only modulates sinusoidal tone (4)(21) but also has the ability to induce choleresis and to stimulate biliary excretion of major organic anions such as glutathione and bilirubin-IXα through mechanisms involving multidrug resistance protein 2 (16). Such effects of CO on biliary excretion occur in a concentration-specific manner, and excess concentrations of the gas repress the choleretic response and lead to cholestasis through the increase in paracellular junctional permeability and suppression of bile canalicular contractility (14, 18). In contrast to CSE, heme oxygenase-1 is up-regulated by surgical insults or by liver cirrhosis and the parenchyma is exposed to high concentrations of CO (5)(10)(12)(24). Thus, under the disease conditions, overproduced CO and reduced H_2S could cooperatively increase the bile acid-independent fraction of bile output through increased excretion of organic anions and HCO_3^-, respectively. Although physiologic implication of the current observation remains to fully be understood, such remodeling of a quality of bile could benefit the increasing solubility of bilirubin and glutathione as anti-oxidants (5) and protect against cholestasis possibly occurring under aforementioned disease conditions. Further investigation is necessary to examine if alterations of these gases could regulate a quality of bile cooperatively with modulation of H_2S generation under a variety of hepatobiliary disease conditions.

Acknowledgments

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Figures and Legends

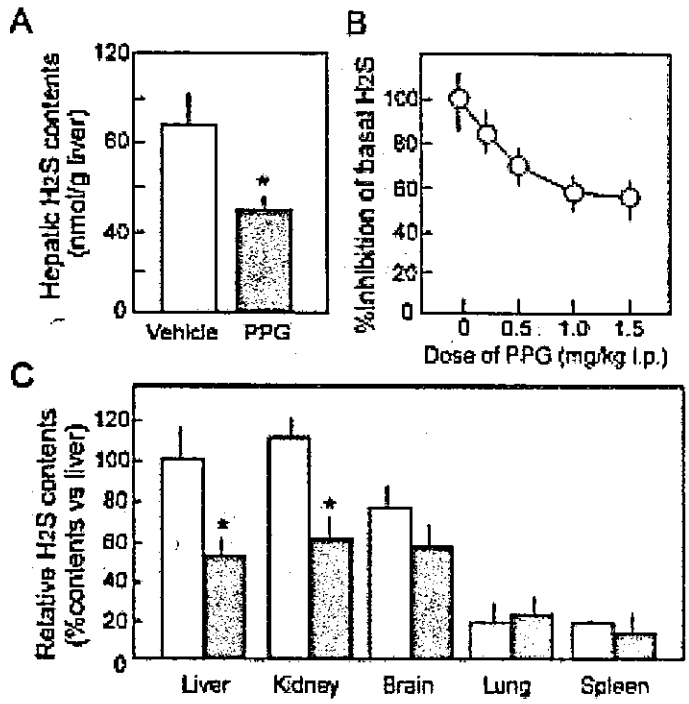

Fig. 1 Effects of administration of propagylglycine (PPG), an inhibitor of cystathionine γ-lyase, on tissue contents of H_2S in vivo. A: The effects of the PPG administration on hepatic H_2S contents. PPG was intraperitoneally injected at 1.5 mg/kg at 4 hrs prior to the experiments. Data indicate mean+/-SE of more than 8 separate experiments. P< 0.05 as compared with the vehicle-treated control group. B: Dose-dependent effects of PPG on the basal H_2S contents in rat livers. C: Differences in the sensitivity to the PPG administration among organs. Open and closed bars represent the tissue H_2S contents in the vehicle- and PPG-treated groups. Data indicate mean+/-SE of 4 separate experiments. P< 0.05 as compared with the vehicle-treated control group.

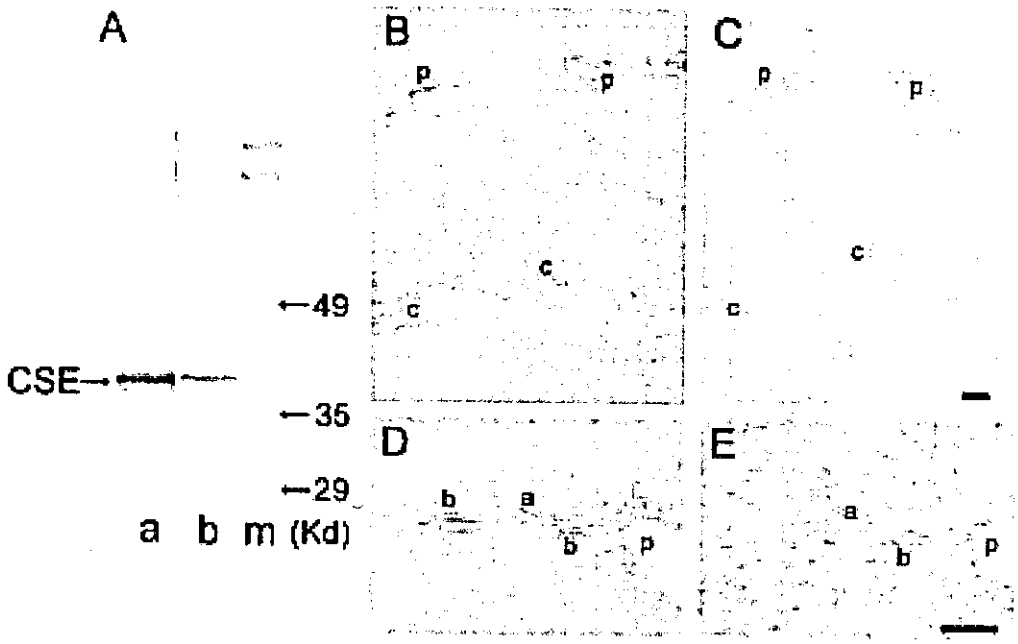

Fig. 2 Expression of cystathionine γ-lyase (CSE) in the rat liver. A: Western blot analyses using the anti-rat CSE antiserum (lane a) and the affinity column-purified antibody (lane b). m: molecular markers. Note a single band in lane b. B and C: Intralobular distribution of CSE in the rat liver stained with the purified anti-CSE Ab and with non-specific chicken IgG, respectively. P and C: portal and central venules. D and E: High magnification of a representative slice stained with the anti-CSE Ab and with the anti-keratin 19 Ab, respectively. a and b: artery and biliary duct. Bar: 50 μm.

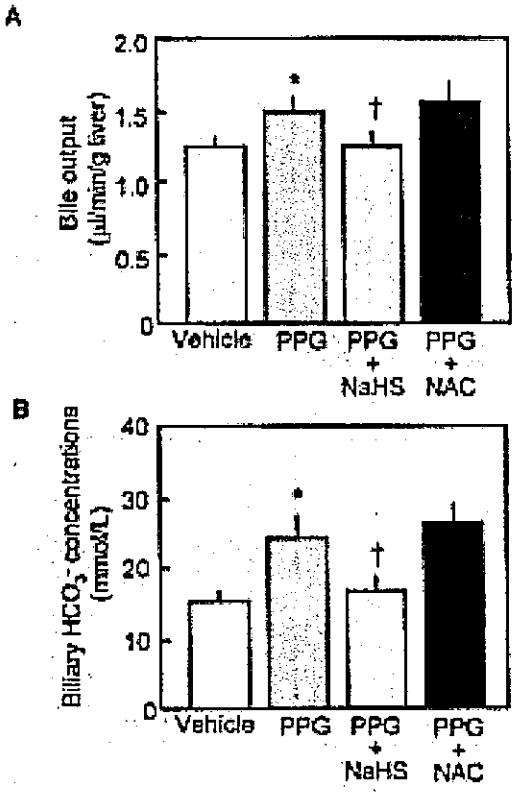

Fig. 3 Effects of the CSE blockade by PPG and supplementation of NaHS on the basal bile output and biliary HCO_3^- concentration in ex-vivo perfused rat livers. PPG at 1.5 mg/kg was administered in vivo intraperitoneally at 4 hrs prior to the isolation of the perfused liver. Either NaHS or N-acetylcysteine (NAC) perfused ex vivo into the liver at a concentration of 30 μmol/L, when necessary. Data indicate mean+/-SE of 7-9 separate experiments in each group. *P<0.05 as compared with the vehicle-treated group. †P<0.05 versus the PPG-treated group.