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Enhancing Effects of Vasoconstrictors on Bile Flow and Bile Acid Excretion in the Isolated Perfused Rat Liver

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ABSTRACT. The effects of vasoconstrictors on bile flow and bile acid excretion were examined in single-pass isolated perfused rat livers. Administration of norepinephrine (NE), 4 nmol/min, plus continuous infusion of taurocholate (TC) (1.0 μmol/min) rapidly increased bile flow in 1 min, and from min 5 until the end of NE administration (late period) bile flow remained above the basal level (111.7 ± 2.2%), as did bile acid output (114.6 ± 1.8%). Without TC infusion, administration of NE produced no increase in the late period. Administration of NE plus taurochenodeoxycholate (1.0 μmol/min) increased bile flow and bile acid output in the late period to 121.9 ± 7.0 and 137.1 ± 6.8%, respectively. With NE plus taurodehydrocholate, the respective values were only 105.4 ± 1.6 and 104.1 ± 4.0%. When horseradish peroxidase (HRP) (25 mg) was infused over 1 min with continuous NE, the late peak (20–25 min) of HRP elimination into bile significantly exceeded that of untreated controls ($P < 0.01$). These observations suggest that vasoconstrictors enhance biliary excretion of more hydrophobic bile acids, in part by stimulating vesicular transport. BIOCHEM PHARMACOL 52;3:489–495, 1996.

KEY WORDS. bile flow; bile acid excretion; vasoconstrictors; intracellular calcium; protein kinase C; vesicular transport

Previous experiments using IPRL† showed that hormones that stimulate intracellular Ca^{2+} transfer (e.g. NE) also increase portal pressure and make liver perfusion uneven because of their vasoconstrictive action. These hormonal actions also decrease bile flow [1–4]. Hormones that elevate the Ca^{2+} level in liver cells are reported to activate PKC [5–7], and PKC activators, such as 12,13-phorbol dibutyrate, increase portal pressure in IPRL and reduce bile flow [6, 8, 9]. Furthermore, the PKC-induced decrease in bile flow occurs even when the elevation in portal pressure is suppressed [10].

Many investigators have reported a reduction in bile flow in the presence of elevated liver cell Ca^{2+} levels. Some representative views are: (1) lithocholate-induced liver cell injury and bile retention are associated with the release of Ca^{2+} from liver microsomes [11, 12]; (2) Ca^{2+} agonists suppress bile flow by elevating liver cell Ca^{2+} levels [8]; (3) elevation in liver cell Ca^{2+} levels enhances permeability across tight junctions, leading to a reduction in bile flow [13, 14]; and (4) stimulation of the α-adrenergic receptors reduces the bile-acid independent bile secretion [15]. Contrary to these views, however, some investigators have described that hormone-induced elevation in liver cell Ca^{2+} levels (1) does not injure liver cells or cause bile retention [16], and (2) causes bile canaliculus contraction, leading to enhanced bile excretion [17, 18]. In addition, it has been found that α-adrenergic agents and vasopressin induce net efflux of the bile salt, TC, from liver cells in suspension [19].

As described above, the influence of elevated liver cell Ca^{2+} levels on bile excretion has not been established unequivocally. Therefore, the present study was performed to clarify the effects of vasoconstrictors on bile flow and bile acid excretion in IPRL. We also assessed the role of the vesicular transport system in the intrahepatocytic transport of bile acid using HRP.

MATERIALS AND METHODS

Animals and Materials

Male Sprague-Dawley rats, 230–290 g, were bred under a normal light–dark cycle. Until the day of the experiment, the rats were allowed free access to a standard solid diet and drinking water. NE, ATII, Arg^8-VP, sodium TC, sodium TCDC, sodium TDHC and HRP (type II) were purchased from the Sigma Chemical Co. (St. Louis, MO). All other

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† *Abbreviations:* IPRL, isolated perfused rat liver; PKC, protein kinase C; HRP, horseradish peroxidase; KRB, Krebs-Ringer bicarbonate; NE, norepinephrine; VP, vasopressin; ATII angiotensin II; TC, taurocholate; TCDC, taurochenodeoxycholate; and TDHC, taurodehydrocholate.

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chemicals were of the highest quality commercially available. The study protocol was approved by the animal care committee of Nagoya City University.

IPRL Studies

IPRLs were prepared as described previously [20]. Abdomens were opened after animals had been anesthetized with sodium pentobarbital (50 mg/kg body weight). In each case after insertion of PE-10 tubing (Clay Adams, Parsippany, NJ) into the bile duct and ligation of the pancreatoduodenal branch of the portal vein, 0.5 mL of sodium heparin was infused into the inferior vena cava. A 16-gauge teflon intravenous catheter (Critikon Inc., Tampa, FL) was then inserted into the portal vein. This was followed immediately by perfusion of the liver with KRB buffer at a rate of 30 mL/min. After isolation, the liver was perfused with this buffer at a constant rate (40 mL/min) using the non-recirculation method, the KRB buffer being warmed to 37° and saturated with a 95% O_2–5% CO_2 mixture. Perfusion pressure was measured by reading the water column pressure, after attachment of an open column to the tip of the portal vein cannula. Perfusion in the untreated controls was performed at a water column pressure of 12–14 cm. Test drugs were infused into the perfusion circuit between the open column and the portal vein cannula by means of a microinfusion pump.

We used three perfusion protocols. With the first, used to examine the influence of vasoconstrictors on bile acid-dependent bile flow, TC dissolved in distilled water was infused continuously at a rate of 1 or 2 μmol/min (concentrations achieved were 25 μmol/L, 50 μmol/L), starting 15 min after beginning the perfusion. Thirty minutes after the flow rate had become stable, 4 nmol/min (100 nmol/L) of NE, 40 pmol/min (1 nmol/L) of ATII, or 40 pmol/min (1 nmol/L) of VP was infused over 15 min. NE was dissolved in saline containing 0.01% ascorbic acid to avoid spontaneous oxidation. After the end of drug infusion, perfusion was continued for 20 min. To examine the influence of vasoconstrictors *per se* on bile flow, a similar experiment was performed without exogenous TC.

With the second protocol, used to examine the influence of vasoconstrictors on various bile acids, NE (4 nmol/min) was administered over 15 min, accompanied by continuous infusion of TCDC or TDHC (each at 1 μmol/min). Both bile flow and bile acid excretion were then assessed.

With the third protocol, used to assess the influence of vasoconstrictors on vesicular transport with HRP serving as an index, NE (1.3 nmol/min), ATII (13 pmol/min), or VP (13 pmol/min) was infused continuously, starting 15 min after the beginning of perfusion. Five minutes after the start of infusion, 25 mg of HRP was infused over 1 min. Bile was sampled several times in the next 50-min period to measure HRP elimination.

Analytic Methods

Bile was sampled on several occasions for determination of bile flow and bile acid excretion. Bile flow was calculated by regarding the weight of 1.0 mL of bile as 1.0 g and was expressed as μL/min/g liver. Total bile acid in bile was quantified by an enzymic method using Enzabile 2 (Daiichi Pure Chemicals, Tokyo, Japan). Lactic dehydrogenase release into the perfusion liquid was also measured enzymatically using the Lactate Dehydrogenase CII-Test (Daiichi Pure Chemicals, Tokyo, Japan). HRP levels in bile were calculated by measuring the 4-aminoantipyrine oxidation rate spectrophotometrically (510 nm).

Statistical Analysis

Data are presented as arithmetic mean ± SEM values. With the third protocol, the area under the HRP secretion curve was calculated as the total HRP output. Because biliary excretion of HRP appeared in two peaks that were separated approximately by 10 min after a pulse load of HRP, the area under the early peak was defined as 0–10 min, and the area under the late peak was defined as 10–50 min. The significance of differences was determined using Student's *t*-test, with $P < 0.05$ regarded as statistically significant.

RESULTS

Effects of Vasoconstrictors on Bile Flow Rate and Bile Acid Excretion (Tables 1 and 2)

Administration of NE (4 nmol/min) accompanied by continuous infusion of TC (1 μmol/min) elevated the portal pressure to 155.6 ± 9.4% of the basal level. Bile flow rate increased to 162.1 ± 12.4% at 1 min, and then decreased. From min 5 to the end of NE infusion (15 min), the bile flow rate remained above the basal level (111.7 ± 2.2%) (Fig. 1). Administration of ATII (40 pmol/min), accompanied by TC infusion (1 μmol/min), increased the portal pressure to 159.3 ± 4.6% of the basal level. Bile flow rate increased to 146.3 ± 5.7% soon after ATII and was still elevated (109.4 ± 0.9%) at 15 min. Administration of VP (40 pmol/min), accompanied by continuous TC infusion (1 μmol/min), produced a slight elevation in portal pressure (102.1 ± 0.3%). Bile flow rate increased rapidly to 174.0 ±

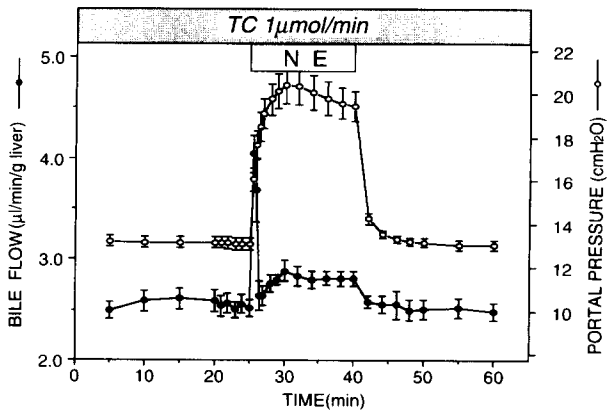

FIG. 1. Effect of NE (4 nmol/min) with continuous infusion of TC (1 μmol/min) on bile flow and portal pressure in IPRL. Data are presented as means ± SEM, N = 5.

TABLE 1. Effects of vasoconstrictors on portal pressure and bile flow

Experimental conditions	Portal pressure		Bile flow	
	Basal (cm H_2O)	Peak (%)	Basal (μl/min/g liver)	Late period (%)
NE alone	13.4 ± 0.2	157.9 ± 7.6*	1.50 ± 0.07	93.1 ± 2.4
ATII alone	13.0 ± 0.2	161.4 ± 8.9*	1.63 ± 0.03	90.9 ± 2.5†
VP alone	13.2 ± 0.2	102.6 ± 0.4	1.62 ± 0.05	91.1 ± 2.9†
NE + TC (1 μmol/min)	13.1 ± 0.3	155.6 ± 9.4*	2.52 ± 0.09	111.7 ± 2.2*
ATII + TC (1 μmol/min)	13.2 ± 0.3	159.3 ± 4.6*	2.48 ± 0.05	109.4 ± 0.9*
VP + TC (1 μmol/min)	13.2 ± 0.2	102.1 ± 0.3	2.39 ± 0.02	109.8 ± 1.7*
NE + TC (2 μmol/min)	13.1 ± 0.2	146.6 ± 2.5*	2.78 ± 0.15	120.2 ± 1.6*
ATII + TC (2 μmol/min)	13.3 ± 0.3	157.1 ± 4.3*	2.80 ± 0.16	118.6 ± 2.6*
VP + TC (2 μmol/min)	12.7 ± 0.3	102.5 ± 0.3	2.68 ± 0.11	115.3 ± 2.5*
NE + TCDC (1 μmol/min)	13.6 ± 0.2	158.2 ± 5.3*	1.34 ± 0.14	121.9 ± 7.0*
NE + TDHC (1 μmol/min)	13.1 ± 0.2	153.5 ± 3.9*	3.30 ± 0.16	105.4 ± 1.6†

Abbreviations: NE, norepinephrine (4 nmol/min); ATII, angiotensin II (40 pmol/min); VP, vasopressin (40pmol/min);TC, taurocholate; TCDC, taurochenodeoxycholate; and TDHC, taurodehydrocholate.

Peak values for portal pressure and average bile flow in the later period (5–15 min) during vasoconstrictor infusion are presented as the percent increase as compared with their basal levels, which are average values for 5 min before vasoconstrictor infusion. Each value represents the mean ± SEM of data for 5 different rats.

* $P < 0.01$.

† $P < 0.05$.

6.1% of the basal level soon after VP and was still elevated (109.8 ± 1.7%) at 15 min. When the administration of vasoconstrictors was accompanied by continuous infusion of TC (2 μmol/min), the elevation in portal pressure and bile flow rate early after their administration resembled those seen with infusion of TC at 1 μmol/min. However, the bile flow rate in the later period during the infusion of vasoconstrictors (5–15 min) exceeded that with the TC infusion rate of 1 μmol/min (120.2 ± 1.6% of the basal level after NE, 118.6 ± 2.6% after ATII, and 115.3 ± 2.5% after VP). The excretion of bile acid 5 min after the administration of vasoconstrictors during the continuous infusion of TC (1 μmol/min) was as follows: 114.6 ± 1.8% of the basal level after NE, 110.7 ± 1.7% after ATII, and 108.9 ± 2.3% after VP. The corresponding values after administration of vasoconstrictors during continuous infusion of TC (2 μmol/min) were 122.7 ± 4.6, 124.8 ± 4.8, and 115.3 ± 3.3%, respectively.

Elevation in portal pressure by each vasoconstrictor was similar both with and without TC infusion. However, although the bile flow rate after NE without concomitant TC infusion rose rapidly to 124.1 ± 5.0% of the basal level, it then fell below this in the later period of NE infusion (93.1 ± 2.4%) (Fig. 2). Similar findings were observed after administration of ATII or VP without the TC infusion.

Effect of NE on Excretion of Various Bile Acids into Bile (Tables 1 and 2)

Since NE, ATII, and VP each had similar effects on bile flow rate and bile acid excretion, we used only NE (4 nmol/min) for assessing the influence of vasoconstrictors on bile acids other than TC. When NE administration was accompanied by continuous infusion of TCDC (1 μmol/min), the bile flow rate increased sharply to 212.1 ± 8.5% of the basal level at 1 min and then decreased, but remained at the later period (5–15 min) above the basal level (121.9 ± 7.0%) (Fig. 3). When NE administration was accompanied by

TABLE 2. Effects of vasoconstrictors on bile acid excretion

Experimental conditions	Bile acid basal level (nmol/min/g liver)	Bile acid in the later period (nmol/min/g liver)
NE + TC (1 μmol/min)	139.7 ± 9.4	161.7 ± 10.8*
ATII + TC (1 μmol/min)	123.6 ± 7.1	136.8 ± 8.2*
VP + TC (1 μmol/min)	129.3 ± 5.9	139.6 ± 4.4†
NE + TC (2 μmol/min)	231.5 ± 14.5	282.0 ± 12.5*
ATII + TC (2 μmol/min)	231.3 ± 13.2	283.3 ± 18.1*
VP + TC (2 μmol/min)	237.1 ± 11.8	271.3 ± 9.9*
NE + TCDC (1 μmol/min)	50.1 ± 6.2	68.7 ± 7.5*
NE + TDHC (1 μmol/min)	281.3 ± 10.1	290.9 ± 5.6

Abbreviations: NE, norepinephrine (4 nmol/min); ATII, angiotensin II (40 pmol/min); VP, vasopressin (40 pmol/min); TC, taurocholate; TCDC, taurochenodeoxycholate; and TDHC, taurodehydrocholate.

Bile acid excretion rate basal levels are average values for 5 min before vasoconstrictor infusion, and those in the later period are average values 10–15 min after vasoconstrictor infusion. Each value represents the mean ± SEM of data for 5 different rats.

*,† Statistically significant difference from basal value: *$P < 0.01$, and †$P < 0.05$.

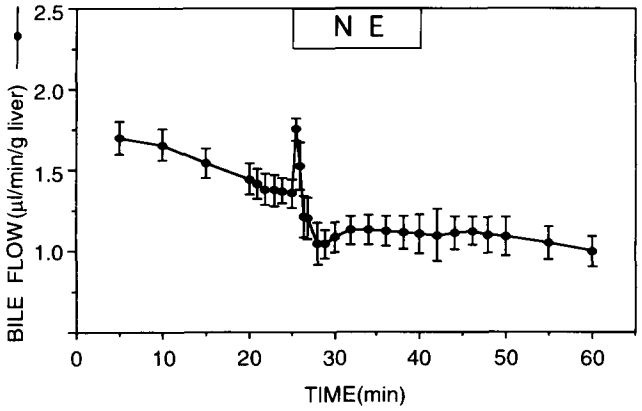

FIG. 2. Effect of NE (4 nmol/min) without bile acids on bile flow in IPRL. Data are presented as means ± SEM, N = 5.

continuous infusion of TDHC (1 μmol/min), the bile flow rate increased to only 121.9 ± 4.2% of the basal level in the early period and was low (105.4 ± 1.6%) in the later period (Fig. 4). Bile acid excretion in the later period after NE administration was 137.1 ± 6.8% when TCDC was infused, but was only 104.1 ± 4.0% of basal level when TDHC was infused.

Effects of Vasoconstrictors on HRP Elimination into Bile (Fig. 5, Table 3)

Vesicular transport, examined by HRP elimination into bile, showed two peaks (an early peak at 4–6 min and a late peak at 20–25 min) in both the untreated control group and the groups given vasoconstrictors. The latter showed significant elevation in the late peak (NE, 25.9 ± 2.99; ATII, 23.1 ± 2.42; VP, 21.7 ± 2.70; control, 8.48 ± 0.46 ng/g liver; $P < 0.01$).

Quality of liver perfusion was judged by the macroscopic appearance of the liver and the degree of lactic dehydrogenase release, no significant increase in this enzyme activity

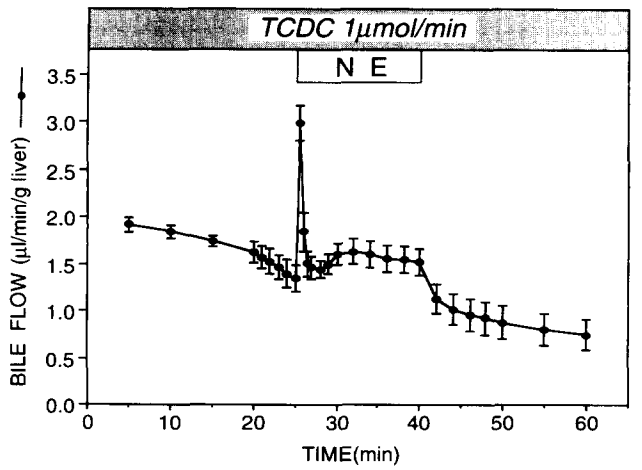

FIG. 3. Effect of NE (4 nmol/min) with continuous infusion of TCDC (1 μmol/min) on bile flow in IPRL. Data are presented as means ± SEM, N = 5.

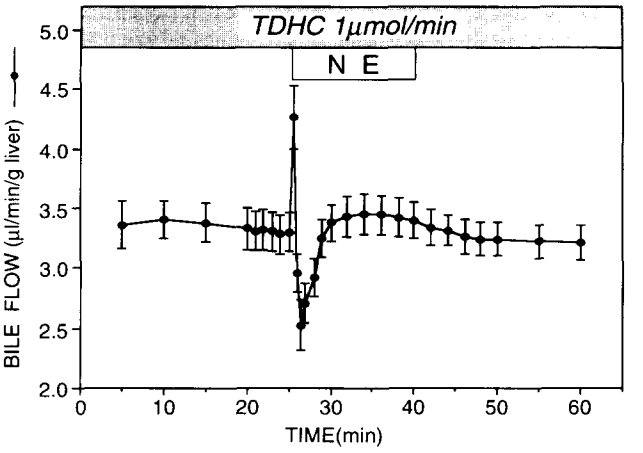

FIG. 4. Effect of NE (4 nmol/min) with continuous infusion of TDHC (1 μmol/min) on bile flow in IPRL. Data are presented as means ± SEM, N = 5.

in the perfusion liquid being seen throughout the experimental course in any case.

DISCUSSION

Treatment of IPRL with vasoconstrictors reduces the bile flow rate because these substances elevate the portal pressure and make liver perfusion uneven [1–4]. Thus, if trypan blue is infused via the portal vein simultaneously with the administration of vasoconstrictors at a concentration high enough to cause a rise of 200% or more in portal pressure, the liver shows an uneven, spotted staining that is accompanied by a reduction in bile flow rate [1]. However, at the concentrations of NE and ATII used in the present study, infusion of trypan blue simultaneously with NE or ATII administration produced an even change in color, suggesting maintenance of homogeneous perfusion of the liver (data not shown). While it is also possible that a moderate elevation in portal pressure can promote the excretion of bile acids, this appears unlikely in our case, since we found that VP, which elevates the portal pressure to only a neg-

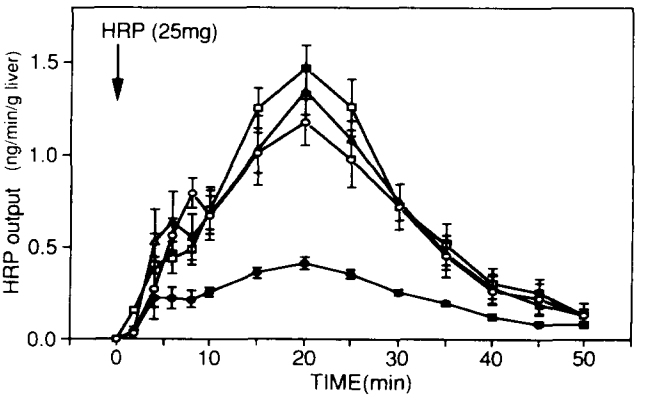

FIG. 5. Effect of vasoconstrictors on the biliary excretion of HRP after pulse loading with HRP. The groups given vasoconstrictors showed a significant elevation ($P < 0.01$) in the late peak. Key: (●) control, (□ NE (1.3 nmol/min), (△) ATII (13 pmol/min), and (○) VP (13 pmol/min). Data are presented as means ± SEM, N = 5.

TABLE 3. Effects of vasoconstrictors on horseradish peroxidase in the early peak (0–10 min) and late peak (10–50 min) in bile

	Horseradish peroxidase (ng/g liver)	
	Early peak	Late peak
Control	1.83 ± 0.31	8.48 ± 0.46
NE (1.3 nmol/min)	3.85 ± 0.68*	25.9 ± 2.99†
ATII (13 pmol/min)	4.29 ± 1.00*	23.1 ± 2.42†
VP (13 pmol/min)	4.11 ± 0.89*	21.7 ± 2.70†

Abbreviations: NE, norepinephrine; ATII, angiotensin II; and VP, vasopressin.
Each value represents the mean ± SEM of data for 5 different rats.
*,† Statistically significant difference from control value: * $P < 0.05$, and † $P < 0.01$.

ligible extent, enhanced bile acid excretion just as did NE and ATII.

In the present study, the administration of vasoconstrictors together with exogenous bile acids resulted in a persistent increase in both bile flow rate and bile acid excretion, but not in their absence, suggesting that these effects are dependent on the presence of bile acids. In contrast, the transient sharp increase in bile flow rate that was observed in the first minute after administration of vasoconstrictors occurred irrespective of the presence or absence of exogenous bile acids and with all types, indicating that this change might result from bile canaliculus contraction [4, 21, 22].

The extracellular Ca^{2+} concentration (in the order of 1 mmol/L) is about 10,000 times higher than the intracellular Ca^{2+} concentration (below 100 nmol/L). Therefore, elevation in intracellular Ca^{2+} and its release from the cells must be controlled to maintain a constant Ca^{2+} level. High concentrations of Ca^{2+} have been shown to be cytotoxic. Moderate elevation and reduction of intracellular Ca^{2+} levels, which occur under physiological conditions, are signals indispensable for intracellular physiological functions. The vasoconstrictor-induced increase in liver cell Ca^{2+} levels seems to involve two mechanisms. When vasoconstrictors bind to receptors, phospholipase C is activated via GTP-bound protein, resulting in the hydrolysis of phosphoinositide-4,5-biphosphate and the formation of inositol-1,4,5-triphosphate (IP_3). IP_3 promotes the release of intracellular Ca^{2+}, resulting in an elevation in liver cytosol Ca^{2+} levels [5, 7, 23]. This increase is followed by inflow of extracellular Ca^{2+} into the cell via Ca^{2+} channels [5, 7, 10]. However, vasoconstrictors are also known to activate PKC [5–7], which reduces bile flow rate in the absence of bile acids in IPRL and isolated rat hepatocyte couplets [6, 8–10].

Although several investigators have described a decrease in bile flow rate associated with a rise in liver cell Ca^{2+} [11–14, 21], such findings were obtained in experiments in which the effects of infusion of exogenous bile acid were not examined. Portal venous blood is known to contain bile acids (*ca.* 40 μmol/L), and since these compounds play an important role in providing a driving force for bile secretion, being efficiently taken up by liver cells and eliminated into bile canaliculi, this is of possible importance. The exact route of bile acid transport within the liver is unknown, but hepatocytes are known to contain three types of cytosolic binding protein (Y, Y′ and Z) [24–29], which might bind to and facilitate their transport to the bile canaliculus.

Some investigators have suggested that vesicular transport is involved in intrahepatocytotic bile acid transport [24–26, 30–32], but intrahepatocytotic bile acid transport takes only 2 min and it is unlikely that vesicles formed by pinocytosis on the sinusoidal side could be transported to the bile canaliculus (a mechanism known for some kinds of protein) within this time. It seems more likely that bile acids are first taken into microsomes and are then only transported via the Golgi apparatus in the form of vesicles to the bile canaliculus [33, 34]. Vesicular transport reportedly becomes a factor in bile acid transport only when large amounts of exogenous bile acids are administered. It is not known to what extent vesicular transport is involved in bile acid transport under physiological conditions [23, 35].

Bolus injection of HRP into IPRL results in the appearance of two peaks of HRP elimination into bile, with the early peak believed to represent paracellular movement or a rapid transcellular microtubule-independent vesicle pathway and the late, predominant, peak considered to reflect a microtubule-dependent vesicle pathway [20, 25, 30, 36–38]. In the present study, administration of vasoconstrictors significantly elevated the late peak, suggesting that vasoconstrictors enhance vesicular transport. The effect of vasoconstrictors in enhancing bile acid excretion was greater in the presence of micelle-forming, hydrophobic bile acids and was negligible in the presence of TDHC, a non-micelle-forming, hydrophilic bile acid. This finding supports the hypothesis that vesicular transport is involved in the intrahepatocytotic transport of hydrophobic bile acids [39].

The molecular mechanism of vasoconstrictor-induced increases in bile flow rate and bile acid excretion observed in the present study is unclear. Bruck *et al.* [9] have reported that VP stimulates the late peak of HRP excretion through activation of PKC, whereas cytosolic Ca^{2+} does not influence HRP excretion. Therefore, the observed increase of bile acid excretion may be caused by PKC activation stimulating vesicular transport.

In our preliminary experiment designed to determine optimum VP levels, the infusion of VP (10 nmol/L) via the portal vein produced only a negligible elevation of portal pressure and an even chromatic response of the liver to trypan blue. However, this treatment reduced the bile flow rate, irrespective of the presence or absence of exogenous bile acids (data not shown). In view of the present findings and those of previous studies [2, 17, 30], it is likely that a high dose of vasoconstrictor suppresses bile secretion, whereas a moderate dose enhances bile acid excretion and elevates bile flow.

In conclusion, the present study demonstrated that vasoconstrictors elevate bile flow in IPRL, with the concentration of vasoconstrictor used and the presence of exog-

enous bile acids being important factors in determining their effects. The mechanism of increased bile acid excretion might conceivably involve stimulation of vesicular transport.

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