Plasminogen Activator in Bile Stimulated by Sodium Taurocholate in Isolated Hamster Livers[1] (34414)

SUSUMU OSHIBA AND LESLIE J. SCHOENFIELD
(Introduced by W. H. Summerskill)

Mayo Clinic and Mayo Foundation, Gastroenterology Unit; Mayo Graduate School of Medicine, University of Minnesota, Rochester, Minnesota 55901

Activity of a plasminogen activator (bilokinase) has been demonstrated in human, bovine, canine, and rabbit gallbladder bile (1, 2). Plasminogen activator is responsible for the conversion of plasminogen to plasmin, a proteolytic enzyme participating in the circulatory fibrinolytic system. Disorders of fibrinolysis occur frequently in diseases of the hepatobiliary system (3), but the origin and the mechanism of secretion of plasminogen activator into bile are not known. The present report describes the effect of sodium taurocholate on the secretion of bilokinase into the bile of the hamster.

Methods. Fibrinolytic activity of bile was measured by the unheated calcium-containing fibrin-plate method described previously (1, 4). This fibrin plate is resistant to the direct lytic action of bile salts. Bile had no observable lytic activity on heated bovine fibrin plates, a fact supporting the contention that the active material is plasminogen activator.

Experiments were done in hamsters about 24 hr after preparation of a bile fistula (gallbladder ligated) and in perfused isolated hamster liver prepared essentially as rat liver was prepared by Brauer and co-workers (5) and by King and co-workers (6). Total bile acids in bile were quantified by the enzymatic method of Talalay (7). The sodium taurocholate (Maybridge Chemical Company, Ltd., Tintagel, N. Cornwall, U.K.) was shown to be 86% pure by thin-layer and gas-liquid chromatography. Secretin was obtained from Vitrum, Stockholm.

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Bile fistulas were prepared in four hamsters. Physiologic saline (0.9%) was infused into the femoral vein at 1.25 ml/hr for 2 hr; then 1% sodium taurocholate was infused at 1.25 ml/hr for 1 hr; and after that saline was again infused for 2 hr. Bile was collected hourly through a polyethylene cannula in the common duct for determination of bile acids and bilokinase.

Bile flow, bile acids, and bilokinase were determined in six experiments with isolated liver, each comprising 4 hr of perfusion dur-

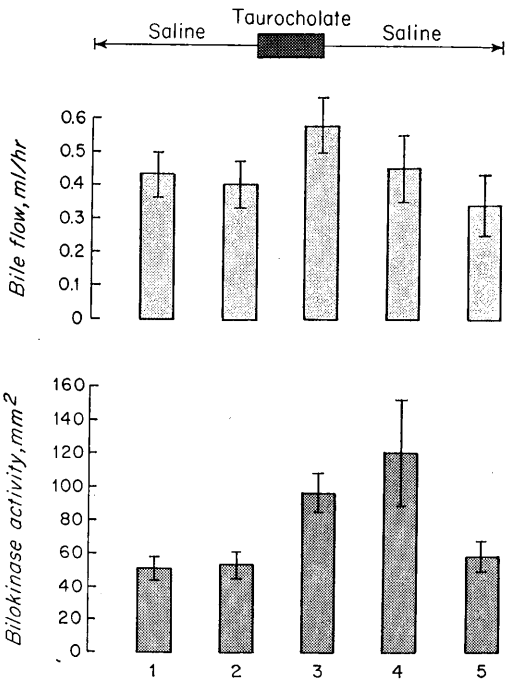

FIG. 1. Stimulation of bile flow and bilokinase in fistula bile of four hamsters during infusion of sodium taurocholate. In Figs. 1–3 and 5 histograms show mean ± SE.

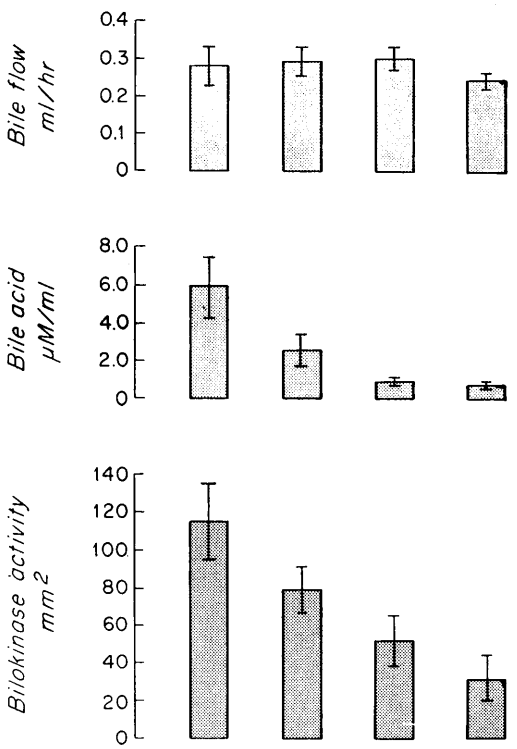

FIG. 2. Bile flow, bile acid, and bilokinase in bile during 4 hr of perfusion in six isolated hamster liver preparations.

ing which bile was collected hourly. In addition, perfusion experiments were done to assess the effect on these indices of sodium taurocholate in seven experiments or secretin in six preparations. At the end of the second hour, 24 μmoles of sodium taurocholate (1% solution) or 0.4 clinical unit of secretion per 100 g of body weight was injected at once into the circulating perfusate (70 ml of hamster blood and 20 ml of saline).

Results. Figure 1 shows data from four hamsters having bile fistulas. During infusion of sodium taurocholate, the bile flow rate ($p<0.05$) and bilokinase activity in bile increased ($p<0.01$). During the 5 hr of control infusions of saline without sodium taurocholate, bile flow and bilokinase remained essentially unchanged.

Figure 2 shows the bile flow, bile acid, and bilokinase in bile during the 4-hr perfusion of six isolated liver preparations. Bile flow remained unchanged during the first 3 hr and decreased slightly though significantly ($p<0.05$) in the fourth hour. The bile acid and bilokinase activity concentrations decreased significantly ($p<0.02$) during each of the first 3 hr of perfusion and remained low during the fourth hour.

Figure 3 shows the effect of taurocholate in seven isolated liver preparations. After the addition of sodium taurocholate to the perfusate, bile flow, bile acid, and bilokinase activity concentrations increased ($p<0.01$). Figure 4 shows a fibrin plate demonstrating the areas of lysis due to bilokinase activity in bile from one isolated hamster liver preparation before and after stimulation with taurocholate.

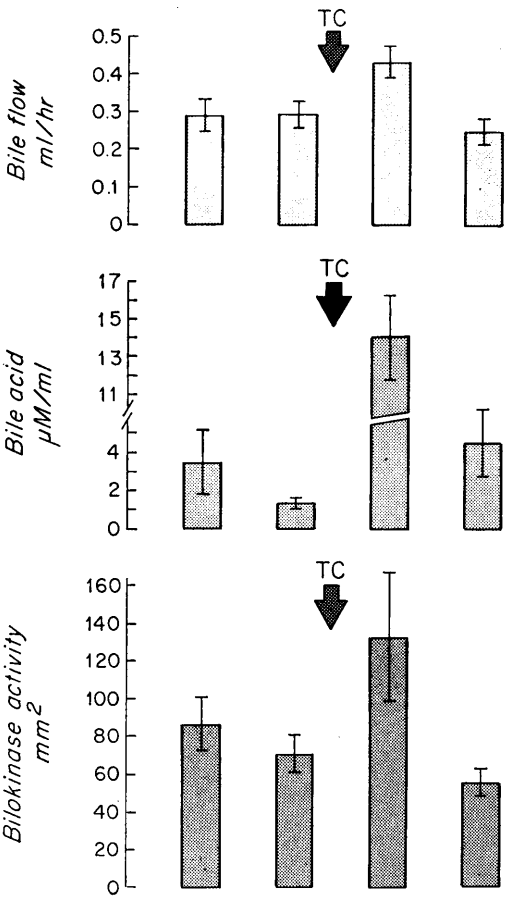

FIG. 3. Stimulation of bile flow, bile acid, and bilokinase in bile of seven perfused isolated hamster livers after injection of 12–15 mg of sodium taurocholate (arrow).

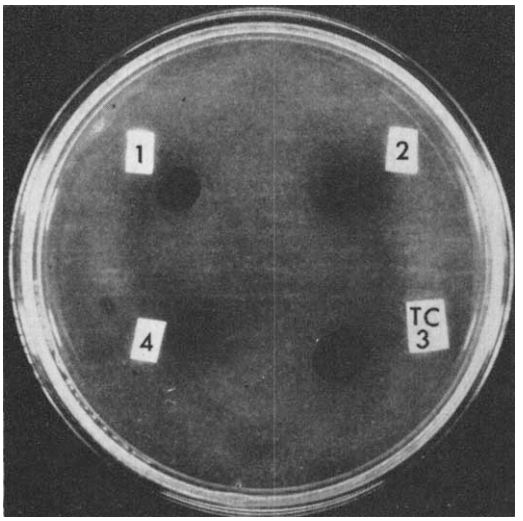

FIG. 4. Unheated fibrin plate showing areas of lysis due to plasminogen-activator activity (bilokinase) from 25-μl aliquots of hourly bile collections (1–4) from isolated hamster liver before and after stimulation with sodium taurocholate (TC).

The effects of secretin on bile flow, bile acid, and bilokinase in bile in six isolated hamster liver preparations are shown in Fig. 5. Secretin resulted in a 23% increase in bile flow ($p<0.05$), but did not alter the bile acid concentration or bilokinase activity.

Comment. Although function of the isolated perfused liver is comparable to that of liver in the intact animals and in those with bile fistula (5, 6), the isolation excludes extrahepatic factors that might affect the biliary secretion of bilokinase. Plasminogen activator activity (bilokinase) has been demonstrated in bile (1, 2), but the mechanism of release into bile and its significance have not been established. Kulapongs and Bachmann (8) demonstrated that rat liver possesses an efficient clearance system for injected urokinase.

Bilokinase in bile may be analogous to urokinase in urine with respect to biologic significance. At least two speculations seem reasonable. Bilokinase may aid in maintaining homeostasis of the coagulation mechanism—which, however, may be altered in hepatobiliary disease (3). Also, just as urokinase has been suggested as helping to prevent formation of renal stones by dissolution of mucosubstances in urine (9), bilokinase may bear a similar relationship to gallstone formation in bile.

The mean increase in bile flow was less after secretin (23%) than after taurocholate (48%). However, those individual experiments wherein secretin choleresis was greatest (and comparable to the results in some taurocholate experiments) were not associated with a significantly increased concentration or output of bilokinase. Hence the increased output of bilokinase into bile after taurocholate is probably the result of a true stimulation or release rather than a so-called washout effect.

The presently reported experiments do not distinguish between an increase in synthesis of bilokinase and an increase in release of it from storage. The solubilizing or dispersing effect of sodium taurocholate on protein mol-

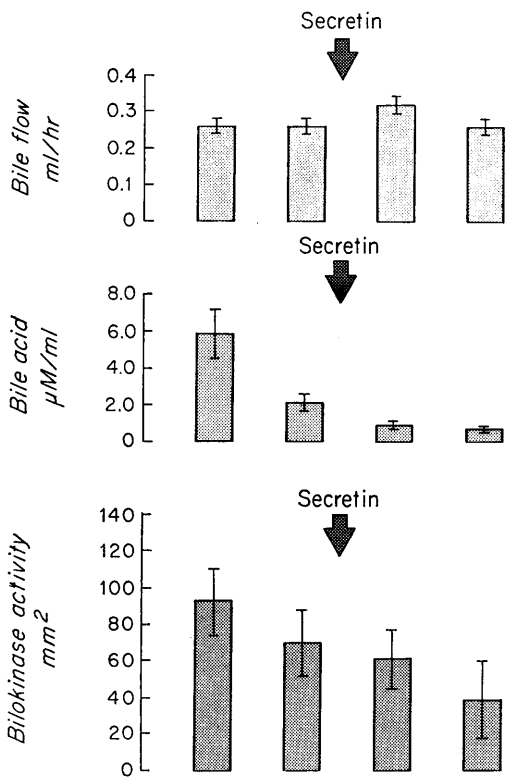

FIG. 5. Stimulation of bile flow by injection of 0.3-0.4 clinical units of secretin/100 g of body weight (arrow) into perfusate of six isolated hamster livers. There was no effect on bile acid concentration or bilokinase activity in bile.

ecules (10) might be responsible for the underlying mechanisms whereby a fixed form of tissue activator in liver is transformed to a soluble form of activator and secreted into bile. Since there was no vasoactive effect of sodium taurocholate in the perfused systems, blood flow probably has no more than a minor influence on the production of this releasable activator.

Summary. Plasminogen activator activity (bilokinase) was demonstrated for the first time in fistula bile of the hamster as well as in the bile of isolated hamster liver. Sodium taurocholate was shown to stimulate the release of bilokinase into bile. Bilokinase in bile is analogous to urokinase in urine, but its role in coagulation homeostasis or in gallstone formation remains to be determined.

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