Table 3.--Morphometric analyses of anatomic changes in the inner stripe of the outer medulla. M ± SEM

	NP rats n = 50 μm^2	LP rats n = 50 μm^2	Significance of difference
Area per vascular bundle	22,800 ± 1,400	23,700 ± 1,000	NS
Thin limb of short loops			
Lumen area	166.8 ± 5.0	144.2 ± 6.3	P<0.01
Epithelium area	34.0 ∓ 1.8	29.2 ∓ 1.6	P<0.05
Thin limb of long loops			
Lumen area	167.5 ± 6.5	178.8 ± 7.8	NS
Epithelium area	74.2 ∓ 4.7	46.7 ∓ 2.8	P<0.001

until more is known about the permeability properties of these nephron segments in LP and NP rats this remains speculation , only. Further interpretation of these results must await additional data. Supported by NIH grant No. RO1 AM 15972.

THE ROLE OF THE ADENYLATE CYCLASE - CYCLIC AMP SYSTEM IN BILE SECRETION BY THE ISOLATED SKATE LIVER

M. Brainard, E.R. Gordon, J.N. Forrest and J.L. Boyer, Department of Medicine, Yale University School of Medicine, New Haven, Ct.

The role of hormones and the adenylate cyclase system in the regulation of biliary secretion is controversial. However, in some mammalian vertebrates, addition of dibutyryl cyclic AMP is associated with a stimulation in bile production (Graf J. Am. J. Physiol. 242:G233-G246, 1983). In this preliminary study, we have examined the effects of forskolin, a diterpene which directly activates the catalytic unit of adenylate cyclase (Seamon, K.B., Daly, J.W.: J. of Cyclic Nucleotide Research 1:201-224, 1981) and 2-chloro adenosine on bile flow in the isolated perfused skate liver. These are specific modifiers of adenylate cyclase activity in a variety of tissues and should therefore be useful in examining if this regulatory enzyme plays a role in bile formation.

Materials and Methods

Livers from the small skate (Raja erinacea) were isolated and perfused with cold oxygenated Elasmobranch Ringers, as previously described (Reed, J.J. et al., Am. J. Physiol. 242:6319-6328, 1982). In each experiment, the gallbladder was opened, the cystic duct cannulated with polyethylene tubing (PE-160) and bile collected in segments of polyethylene tubing (PE-90). Volume per unit length of tubing was calculated and, at 10 or 15 minute intervals, marks were made on the collection tube to record the rate of bile production. Bile flow was monitored for 1 to 2 hours until a stable baseline rate of secretion was established. In twelve studies, forskolin (5 μM or 0.05 μM) or dibutyryl cAMP (10 μM) was added to the system. After 1-2 hours, the perfusate was then exchanged with fresh Elasmobranch Ringers, to remove these agents and bile flow was monitored for an additional 1-2 hours.

In a second set of 5 experiments, the Ringer's solution was perfused in a single pass through the liver. After a baseline secretion rate had been established, the liver was alternately perfused (for approximately half-hour periods) with 1 μM 2-chloro adenosine, 1 μM 2-chloro adenosine plus 10 μM theophylline, or Elasmobranch Ringers alone while bile flow was continuously recorded.

Results and Discussion

Treatment with 5 μM forskolin inhibited bile secretion in all experiments (43 ± 22% of control; n = 5), and subsequent perfusion with forskolin-free ringers led to a significant recovery (80 ± 37% of control). Figure 1 illustrates a representative experiment. Reducing the concentration of forskolin to 0.05 μM abolished its inhibitory effect. Only one of five experiments showed significant depression of bile secretion from its control value, while in two ex-

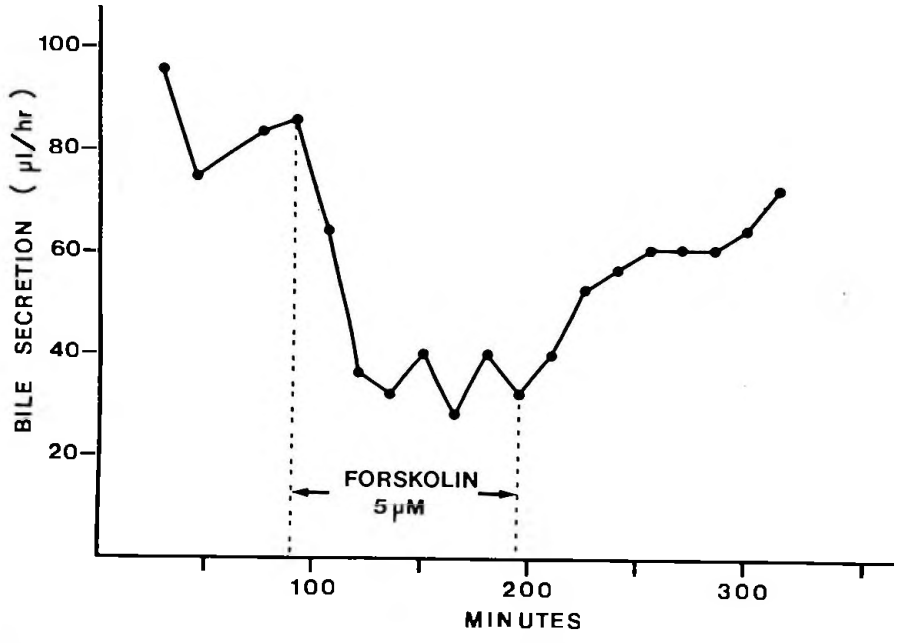

Figure 1.--The effect of forskolin (5 µM) on bile secretion by the isolated liver of the small skate.

periments there was a marked increase, suggesting that the low dose of forskolin may have stimulated secretion in these experiments. During these experiments perfusion pressures were also monitored, as it has been previously demonstrated that bile secretion in the elasmobranch is in part dependent on perfusion pressure (Reed J.J., et al., Am. J. Physiol., 242:6319-6328, 1982). However, changes in portal pressures were minimal and could not account for the observed changes in biliary flow during the administration of forskolin.

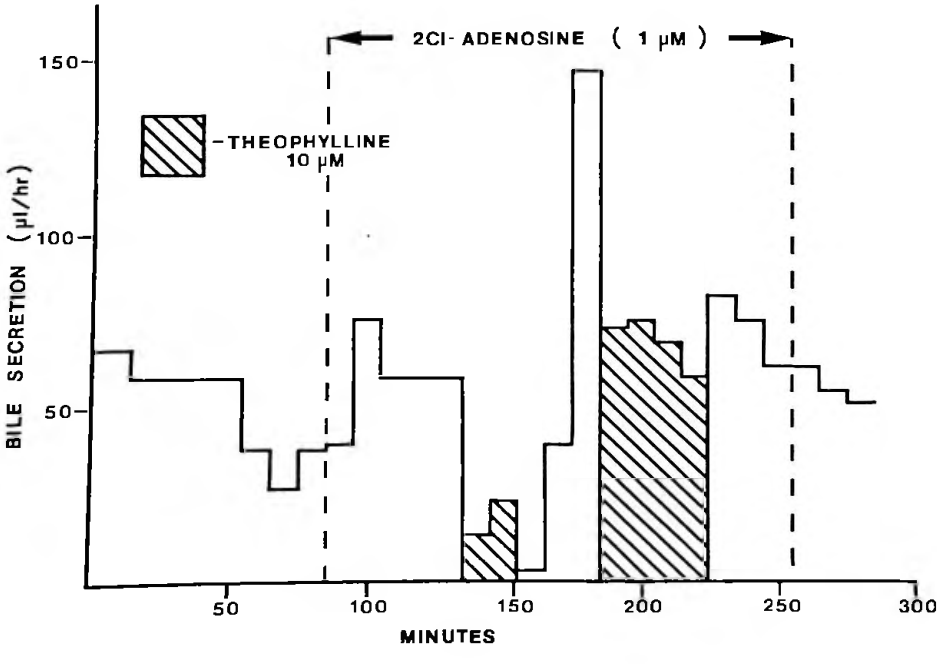

Figure 2.--The effect of 2-chloro adenosine and theophylline on bile secretion by the isolated liver of a small skate. The 2-chloro adenosine level in the non-recirculating perfusate was maintained at 1 µM from 85 to 255 minutes. Theophylline (10 µM) was added to the perfusate at the times indicated.

When 10 μM dibutyryl cAMP was administered to the perfusate (2 studies only) bile secretion was significantly inhibited to flow rates that were 30% and 50% of control values. However, there also was no significant recovery following perfusion with dibutyryl cAMP-free ringers. The effect of forskolin and dibutyryl cAMP are opposite to that observed in mammalian systems where similar concentrations of dibutyryl cAMP have been shown to stimulate bile secretion (Graf, J., In: Stimulus Secretion Coupling in the Gastrointestinal Tract, ed. by R.M. Case and H. Goebell, pub. University Park Press, Baltimore, p. 301, 1976).

Two types of membrane bound adenosine receptors have previously been identified in many tissues including the liver. The activation of these receptors enhances or reduces intracellular levels of cyclic AMP (Londos, C., et al., Proc. Nat'l. Acad. Science U.S.A., 77:2551, 1980). The elasmobranch (dogfish) rectal gland appears to contain adenosine receptors both stimulating and inhibitory for adenylate cyclase (Forrest, J.N., et al., Bull. MDIBL 20:152-155, 1980; Forrest, J.N., et al., Bull. MDIBL 22:51, 5-15, 23, 1982). In three out of five experiments in which we administered 1 μM 2-chloro adenosine (the slower metabolized analogue of adenosine) bile secretion was significantly greater than control (257 ± 151%). Furthermore, the simultaneous addition of 10 μM theophylline, which competes for the adenosine receptor, reduced secretion in each of the experiments where stimulation was observed (47 ± 23.5% of the stimulated rate). In one experiment, both stimulation and inhibition of secretion could be reproduced in the same liver (Fig. 2).

These preliminary studies indicate that cAMP may play a role in regulating the secretion of bile in the small skate. Further studies are required to define the sensitivity of this system to hormones and the mechanisms involved. Since these effects are observed in an isolated portal vein perfused liver system, the site of the cyclase effect is likely to be the hepatic parenchymal cell. However, these agents may have altered transport systems in the bile ducts although these structures are primarily perfused via the hepatic artery which is not functional in this system.

Supported by USPHS, Grant No. 25636.

THE SECRETION PATTERN OF TETRAPYRROLES IN THE SMALL SKATE (RAJA erinacea)

M. Grossbard, J.L. Boyer and E.R. Gordon, Department of Medicine, Yale University, School of Medicine, New Haven, Ct.

Unconjugated bilirubin, the conjugates of bilirubin, and biliverdin have been detected in gallbladder bile of the small skate (Raja erinacea) (Chowdhury et al., Comp. Biochem. Physiol. 66B:523-528, 1980; McDonagh and Palma, Comp. Biochem. Physiol. 73B:501-507, 1982). The question then arises as to the source of the biliverdin appearing in Elasmobranch bile. The biliverdin could be an end product of heme metabolism or it could be formed by the oxidation of bilirubin in the gallbladder. This question was investigated in the following studies by comparing the tetrapyrrole composition of the gallbladder bile and hepatic bile of the small skate.

Methods

Animals--Forty-two small 1 kg male skates (Raja erinacea) were obtained by net in waters off Southwest Harbour, Maine during the summer of 1983, and maintained in well-oxygenated tanks at Mount Desert Island Biological Laboratories until they were used. Gallbladder bile was obtained by aspiration and hepatic bile was obtained in free swimming fish from a cannula inserted into the gallbladder as previously described (Boyer et al., Am. J. Physiol. 230:970-973, 1976).

Analytical Procedures--The volume of bile was measured, and ascorbic acid (5 mg/ml) was added to the samples which were protected from light and stored frozen until analyzed. Extraction, detection and quantitation of the tetrapyrroles of bilirubin and biliverdin were carried out as described in the literature (McDonagh et al. Comp. Biochem. Physiol. 73B:501-507, 1982; Gordon et al. Can. J. of Biochem. 60:1050-1058, 1982).