Occurrence of α and β Receptors in the Bile Duct. (30473)

A. CREMA, G. BENZI, G. M. FRIGO AND F. BERTÈ (Introduced by C. A. Winter)
Department of Pharmacology, University of Pavia, Italy

From previous studies concerning the action of some sympathomimetic drugs *in vitro* and *in situ*, we postulated that in the terminal bile duct there are α-receptors, whose activation causes a constriction, and β-receptors, whose activation causes a dilatation(1,2). As there are also α- and β-receptors in the duodenum, it was impossible to block the receptors of one organ leaving unaffected those of the other, if the bile duct were allowed to remain imbedded in the duodenum.

Recently we described a method for testing in the guinea pig the selective action of the drugs upon the biliary tract(3). In fact in the guinea pig the greatest part of the terminal bile duct is contiguous with and not inside the duodenum; therefore, it is easy to pull out the Vater's ampulla from the duodenum. By this method, which allows one to record separately *in situ* the response of the terminal bile duct and of the duodenum, we studied the action of some sympathomimetic and sympatholytic agents.

Method. The method described elsewhere (3) is briefly summarized as follows: 128 guinea pigs were used; animals anesthetized with urethane (1 g/kg i.p.) plus chloralose (20 mg/kg i.p.) were given artificial respiration; blood pressure was measured from a cannula inserted into a carotid artery. After laparotomy, an inflow-tube was inserted through the gall bladder into the cystic duct and tied below the Lütkens' area. The hepatic ducts were tied caudally and partially excised; hepatic bile was removed by means of a pump.

The terminal choledochus was freed from the peritoneum joining it to the duodenum and gently pulled out from the duodenal musculature. The flow through the bile duct was regulated by adjusting the height of a Mariotte bottle containing Tyrode solution with or without drugs; the flow was measured by a drop-counter inserted between the bottle and the duct. The Tyrode's solution flowing through the sphincter of Oddi was drained outside the abdomen by a funnel-shaped collecting tube. Movements and tone of the duodenum and of the jejunum were recorded by means of 2 water-filled rubber balloons.

Drugs were perfused through the bile duct in Tyrode solution at the following concentrations: dibenamine hydrochloride 10-20 μg/ml; pronethalol 10-20 μg/ml; (—)epinephrine hydrochloride 25-50 μg/ml; (±)isoprenaline hydrochloride 25-50 μg/ml; (—) phenylephrine hydrochloride 25-50 μg/ml.

Results. Intravenous injection of epinephrine (0.5-5 μg/kg) induced a decrease in the flow through the bile duct (Tables I and III). This could be observed even when the tone of Oddi's sphincter was artificially increased by administration of sub-maximal doses of parasympathetic drugs. Similarly, intravenous injection of phenylephrine (5-25 μg/kg) decreased biliary flow, while intravenous injection of isoprenaline (0.5-5 μg/kg) increased flow through the bile duct (Tables I and III). Epinephrine induced a decrease in the tone of the duodenum and an increase in tone and motility of the terminal portion of the jejunum. The response of Oddi's sphincter to epinephrine did not appear to be related to the change in blood pressure. In fact, under normal blood pressure conditions, by injecting a quantity of glucose solution (5%) or of epinephrine in a dose producing a similar blood pressure increase, the sphincter spasm was much greater after administration of the latter.

Local perfusion of epinephrine or of phenylephrine (25-50 μg/ml of Tyrode solution) through the biliary tract induced a constriction in the terminal portion of the choledochus without changing the blood pressure or the tone of the intestine. The introduction of isoprenaline in the Tyrode solution (25-50 μg/ml) increased the flow through the terminal tract of the choledochus (Table II). The rather high concentrations of drugs necessary when applied into the lumen of the bili-

TABLE I. Intrabiliary Perfusion with the Blocking Drugs: Mean Flow (ml/Min ± S.E.) Calculated During 4 Min Before and After the Sympathomimetics Injected Intravenously. n = No. of animals.

Control flow, ml/min	Sympathomimetic drugs: Drug	Dose i.v., μg/kg	n	Flow after intrabiliary perfusion with: —	n	Dibenamine, 20 μg/ml	n	Pronethalol, 20 μg/ml
	Epinephrine	1.0	8	.08 ± .027	5	.10 ± .009	3	.08 ± .009
		2.0	8	.06 ± .015	5	.12 ± .006	3	.05 ± .009
		4.0	7	.03 ± .004	4	.11 ± .010*	3	.02 ± .006
.11 ± .014	Phenylephrine	5.0	6	.10 ± .021	4	.12 ± .019	2	.11 ± .010
		10.0	7	.08 ± .008	4	.10 ± .016	3	.09 ± .008
		20.0	7	.05 ± .005	4	.11 ± .011	3	.06 ± .010
	Isoprenaline	1.0	7	.26 ± .012	3	.28 ± .036	4	.12 ± .009
		2.0	7	.33 ± .020	3	.34 ± .035	4	.10 ± .012
		4.0	7	.41 ± .021	3	.41 ± .029	4	.11 ± .012

* Not included 2 animals in which there was a reversal.

ary tract may be related to low permeability of the choledochus (Eisenstein, 4).

By perfusing dibenamine (10-20 μg/ml of Tyrode solution for 30 minutes) through the biliary tract, we observed a blockage of the action of epinephrine (0.5-5 μg/kg i.v.) and phenylephrine (5-25 μg/kg i.v.) on the biliary tract, without affecting the response of the blood presure and the intestine. In 2 animals Oddi's sphincter showed a reversal to epinephrine after dibenamine. The response to intravenous administration of isoprenaline (0.5-5 μg/kg) was unaffected by local perfusion of dibenamine (Table I). By perfusing locally pronethalol (10-20 μg/ml of Tyrode solution for 30 minutes) through the biliary tract, we noted a blockage of isoprenaline action only on the Oddi's sphincter, while the response to epinephrine is hardly affected and that to phenylephrine remains unchanged (Table I).

TABLE II. Intrabiliary Perfusion with Sympathomimetic Drugs: Mean Flow (ml/Min ± S.E.) Calculated During 4 Min Before and After the Perfusion with Sympathomimetics. n = No. of animals.

Control flow, ml/min	Drug	Local conc, μg/ml	n	Flow
	Epinephrine	25	4	.08 ± .046
		50	4	.06 ± .015
.12 ± .003	Phenylephrine	25	4	.10 ± .029
		50	4	.09 ± .010
	Isoprenaline	25	4	.17 ± .013
		50	4	.24 ± .036

The action of the blocking agents is similar either if injected i.v. (Table III) or perfused into the biliary tract (Table I).

Also 60 minutes after washout the action of dibenamine was not reversed, while reversibility was a common occurrence for pronethalol.

Summary and conclusion. By means of a new method which permits one to evaluate separately the behavior *in situ* of the chole-

TABLE III. Systemic Treatment with the Blocking Drugs: Mean Flow (ml/Min ± S.E.) Calculated During 4 Min Before and After the Sympathomimetics Injected Intravenously. n = No. of animals.

Control flow, ml/min	Sympathomimetic drugs: Drug	Dose i.v., μg/kg	n	Flow after intravenous injection of: —	n	Dibenamine, 10 mg/kg	n	Pronethalol, 2.5 mg/kg
	Epinephrine	2.0	4	.07 ± .011	2	.12 ± .007	2	.05 ± .020
		5.0	4	.02 ± .003	2	.16 ± .020	2	.02 ± .010
.12 ± .005	Phenylephrine	10.0	4	.07 ± .005	2	.11 ± .015	2	.07 ± .010
		25.0	4	.05 ± .008	2	.12 ± .020	2	.06 ± .010
	Isoprenaline	2.0	4	.35 ± .030	2	.37 ± .030	2	.11 ± .020
		5.0	4	.44 ± .037	2	.45 ± .050	2	.12 ± .030

dochus and duodenum of guinea pig, we confirmed our previous assumption concerning the existence in the terminal bile duct of α-receptors whose activation caused a constriction and β-receptors whose activation caused a dilatation. The contracting action of epinephrine was selectively blocked by dibenamine, while the relaxing action of isoprenaline was blocked by pronethalol.

1. Crema, A., Bertè, F., Brit. J. Pharmacol., 1963, v20, 221.
2. Benzi, G., Bertè, F., Crema, A., Frigo, G. M., ibid., 1964, v23, 101.
3. Crema, A., Benzi, G., Frigo, G. M., Bertè, F., J. Pharm. Pharmacol, in press.
4. Eisenstein, M. W., Surg. Gyn. Obst., 1949, v89, 49.

Received April 7, 1965. P.S.E.B.M., 1965, v120.

A Possible Mechanism for the Anti-Inflammatory Effects of Proteolytic Enzymes.* (30474)

W. L. GABLER†‡ AND L. S. FOSDICK (Introduced by N. M. Phatak)
Chemistry Department, Northwestern University Dental School, Chicago, Ill.

A number of investigators have reported that certain of the proteolytic enzymes possess anti-inflammatory properties(1,2,3). Innerfield(4) suggests that an enzyme may act as an anti-inflammatory agent by nature of its enzymatic properties, provided it is in an active form in the edema fluid. Among the functions of enzymes in the role of an anti-inflammatory agent, according to Innerfield, are hydrolysis of macromolecules (thus easing their removal), the lowering of the viscosity of the edema fluid and the breakdown of lymphatic blockades.

The present work was undertaken to see if enzymes injected at a point distant to the site of inflammation can be found in an active form in the edema fluid. A second phase of the work was to note the effect of a proteolytic enzyme on the flow rate and composition of lymph derived in part from the area of inflammation. The effects of an enzyme on the volume and protein composition of the lymph and edema fluid were also studied. A comparison of the changes in lymph and edema fluid under enzyme influences may shed some light on the mechanism of their anti-inflammatory activities.

The pleural space was selected as the site to induce inflammation since both edema and lymph derived from this space can be collected in quantities sufficient for analysis and relatively free from tissue contamination. Lymphatic drainage from the pleural space is channeled, in part, into the thoracic duct where it can be collected for analysis(5).

Method. Male albino rats weighing 350-390 g were used in this study. The rats were lightly anesthetized with ether; 5 ml of a solution containing 1% gum arabic in saline were injected into the right pleural space of the rat by means of a 5-mm-long #26 needle. Concomitantly, 1 ml of the test enzyme solution was injected subcutaneously into the left hind leg above the knee joint. The enzyme was dissolved in 1 ml of saline and brought to body temperature immediately prior to use. Controls received leg injections of 1 ml of saline without the enzyme.

After appropriate time intervals the rats were sacrificed with ether, pleural cavities opened and the fluid withdrawn. The volume of fluid collected was measured in a graduated centrifuge tube, allowed to clot and stored at 4°C. The clotted material was centrifuged at 2000 rpm and the supernatant was used for analysis.

To study the effect of chymotrypsin on the

* This study was supported in part by grant HF-12,422-C1, Nat. Inst. Health, U.S.P.H.S.

† Taken in part from a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree in chemistry at Northwestern University, Evanston, Ill., Aug. 1964.

‡ Present address: Biochemistry Dept., University of Oregon Dental School, Portland.