Br. J. Pharmac. (1982) **77**, 717–723

STIMULATORY AND INHIBITORY HISTAMINE RECEPTORS IN CANINE CYSTIC DUCT

A.S. CLANACHAN, D.F. COURTNEY* & G.W. SCOTT*

Biliary Research Group, Departments of Surgery* and Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2N8

1 The effects of histamine receptor stimulation were assessed on the resistance of the canine cystic duct *in vivo* and on the contractility of circular muscle preparations of canine cystic duct *in vitro*.

2 In anaesthetized dogs, the H_1-receptor agonist, 2-pyridylethylamine (0.05 to 15 μmol, i.a.), elicited dose-dependent increases in cystic duct resistance, whereas the H_2-receptor agonist, 4-methylhistamine (0.05 to 15μmol, i.a.) decreased cystic duct resistance. These responses were antagonized by the H_1-receptor antagonist, diphenhydramine, and the H_2-receptor antagonist, cimetidine, respectively.

3 Histamine (0.1 to 3000 nmol, i.a.) also increased cystic duct resistance *in vivo*. In the presence of diphenhydramine, the stimulatory effect of histamine was antagonized and slight decreases in cystic duct resistance became apparent. Cimetidine or prazosin also antagonized the stimulatory effects of histamine.

4 Histamine (1 to 100 μM) or 2-pyridylethylamine (1 to 100 μM) contracted, whereas 4-methylhistamine (1 to 100 μM) relaxed, circular muscle preparations of cystic duct. These excitatory and inhibitory responses were antagonized by diphenhydramine and cimetidine, respectively.

5 These results indicate that the canine cystic duct possesses excitatory H_1- and inhibitory H_2-receptors. The predominant effect of histamine is an H_1-receptor-mediated increase in cystic duct resistance. Histamine, which may be released in association with cholecystitis, may exert significant effects on the regulation of bile flow in and out of the gallbladder and may contribute to gallbladder stasis during biliary disease.

Introduction

The cystic duct is approximately 1.5 cm long and joins the gallbladder to the common bile duct. It conveys bile to the gallbladder during fasting periods and out of the gallbladder after meals. There is a thin layer of muscle in the wall of the duct, and the duct appears to have sphincter-like properties. Potter & Mann (1926) observed that the pressures within the gallbladders and common bile ducts of conscious dogs could vary independently of each other, and this was confirmed by Doyle & Farrar (1969). Scott & Otto (1979) found that the resistance to flow through the canine cystic duct increased after systemic or local intra-arterial bolus injections of morphine or cholecystokinin (CCK). Similarly, the flow of contrast medium through the human cystic duct, observed by using a cine-radiographic method, was reduced after systemic administration of morphine or CCK (Torsoli, Ramorino & Alessandrini, 1970). We have recently established (unpublished) both *in vivo* and *in vitro* that stimulation of α-adrenoceptors or muscarinic receptors constricts the canine duct whereas stimulation of β-adrenoceptors relaxes the duct. No information is available regarding the presence of histamine receptors in the cystic duct.

Histamine is present in mast cells and most connective tissues throughout the body and its effects are mediated by two types of receptors. For example, stimulation of H_1-receptors causes contraction of the smooth muscle of the gut and bronchi (Ash & Schild, 1966) and stimulation of H_2-receptors increases gastric acid output and increases heart rate (Black, Duncan, Durant Ganellin & Parsons, 1972). Thus, the overall effects of histamine in a tissue will depend on the relative numbers and distribution of the two types of receptors in that tissue. Stimulatory H_1- and inhibitory H_2-receptors have been identified in gallbladder muscle (Waldman, Zfass & Makhlouf, 1977; Gadacz, 1978; Impicciatore, 1978), and histamine acting on H_2-receptors is capable of relaxing the sphincter of Oddi (LaMorte, Gaca, Wise, Birkett & Williams, 1980) at the terminal end of the common bile duct. The present investigation examines the

0007-1188/82/120717-07 $01.00

effects of stimulation of H_1- and H_2-receptors on cystic duct resistance *in vivo* and on the contractility of cystic duct strips *in vitro*.

Methods

Measurement of cystic duct resistance in vivo

Acute experiments were performed on healthy male or female mongrel dogs, each weighing approximately 20 kg. They were fasted overnight and then anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.). Body temperature was maintained with warming pads and a heat lamp. The abdomen was opened via a midline incision and the gallbladder, cystic duct and common bile duct were identified. A specially constructed metal inflow catheter was passed through a stab-wound in the gallbladder and anchored with a tie so that its orifice was 2 mm within the cystic duct (Figure 1). The accurate placement of this catheter was confirmed at the conclusion of each experiment. This inflow catheter was connected to a saline reservoir (Figure 1), the height of which could be adjusted, via an electromagnetic flowmeter (Zapeda SWF-1M), which recorded the flow of saline, and a heating coil (37°C). The inflow catheter (2.5 mm i.a.) contained a separate fine metal cannula (0.5 mm i.d.) which was connected to a pressure transducer (Statham P2310) for the measurement of perfusion pressure of the saline solution.

A metal outflow catheter (3.5 mm i.d.) was inserted through a stab-wound into the common bile duct and positioned so that its tip was adjacent to the end of the cystic duct. This catheter provided a low resistance outflow for the saline solution plus the bile which was being secreted continuously by the liver. The cystic duct perfusion pressure was initially adjusted to zero and then slowly raised (to 5–7 cmH_2O) until a perfusion flow of 11 $ml\,min^{-1}$ was obtained. This flow rate was determined initially to be optimal for the measurement of changes in cystic duct resistance.

The gastroduodenal artery was cannulated retrogradely to permit the administration of drugs into the hepatic artery. Changes in flow through the cystic duct were taken to reflect changes in cystic duct resistance and responses were assessed as the maximal change in flow ($ml\,min^{-1}$) from control (11 $ml\,min^{-1}$). Perfusion flow was allowed to return

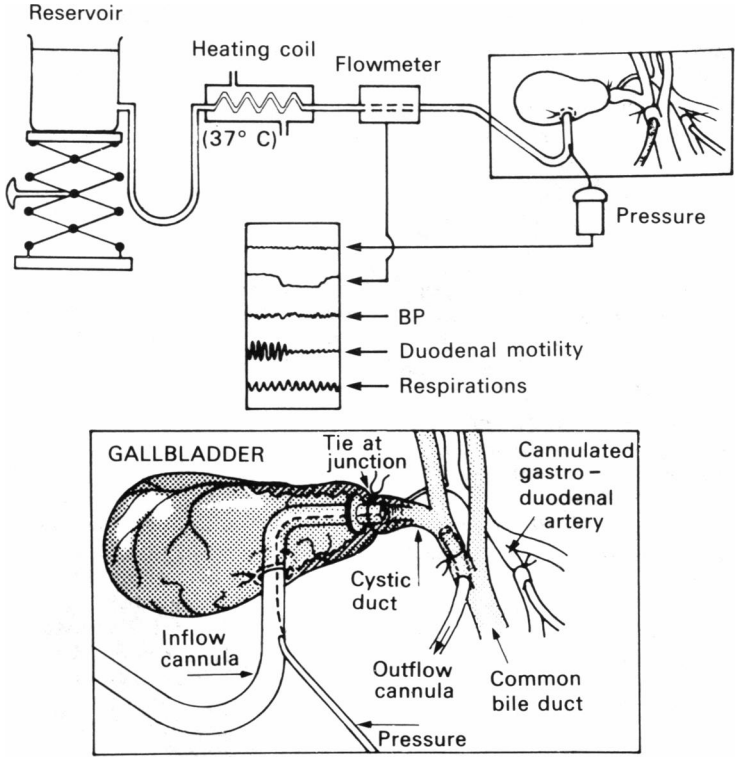

Figure 1 Diagrammatic representation of the method used to determine drug-induced alterations in the resistance of the canine cystic duct. See text for details.

to control values between doses. Recordings were also made of systemic arterial pressure (via a cannulated femoral artery), duodenal motility (duodenal strain gauge transducer) and respiratory movements (pneumatic cuff). Graded doses of the various histamine receptor agonists were administered into the hepatic artery in unblocked (control) dogs and then, in the same animal, following the intravenous administration of the appropriate receptor antagonist. In an initial series of experiments ($n = 3$), a second dose-response curve prepared in the same, unblocked animal was not significantly different from the initial control. This indicated that there were no time-dependent changes in the responsiveness of the cystic duct during the course of an experiment.

Measurement of cystic duct motility in vitro

Gallbladders and cystic ducts were removed from dogs anaesthetized with pentobarbitone. Tissues were opened along their longitudinal axes and washed in oxygenated Krebs solution. Each cystic duct was divided transversely into four portions (2 × 8 mm) and these were suspended in organ baths (5 ml) containing Krebs solution at 37°C and gassed with 95% O_2/5% CO_2. The resting tension was adjusted to 500 mg and strips were allowed to stabilize for 30 min. Contractions of the tissues were recorded isometrically with force displacement transducers (Grass FT. 03C) and displayed on a polygraph recorder. Concentration-response curves were prepared for the various histamine receptor agonists. Histamine receptor antagonists, where appropriate, were usually added 30 min before determination of agonist concentration-effect curves. Responses are expressed as a percentage of the maximal contraction or relaxation of each strip in response to a supramaximal concentration of acetylcholine (ACh 1 mM) or isoprenaline (100 μM), respectively.

Chemicals and drugs

The Krebs solution had the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$, 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22 and D-glucose 11.2. The solution (pH = 7.4) was equilibrated with 95% O_2 and 5% CO_2 and maintained at 37°C.

Drugs used, and their sources, were: acetylcholine chloride, atropine sulphate, diphenhydramine hydrochloride, histamine diphosphate (purchased from Sigma Chemical Co.); cimetidine hydrochloride, 2-pyridylethylamine dihydrochloride, 4-methylhistamine dihydrochloride, (donated by Smith, Kline & French (Canada) Ltd); prazosin hydrochloride, (donated by Pfizer, Canada); sodium pentobarbitone, (purchased from MTC Pharmaceuticals Ltd).

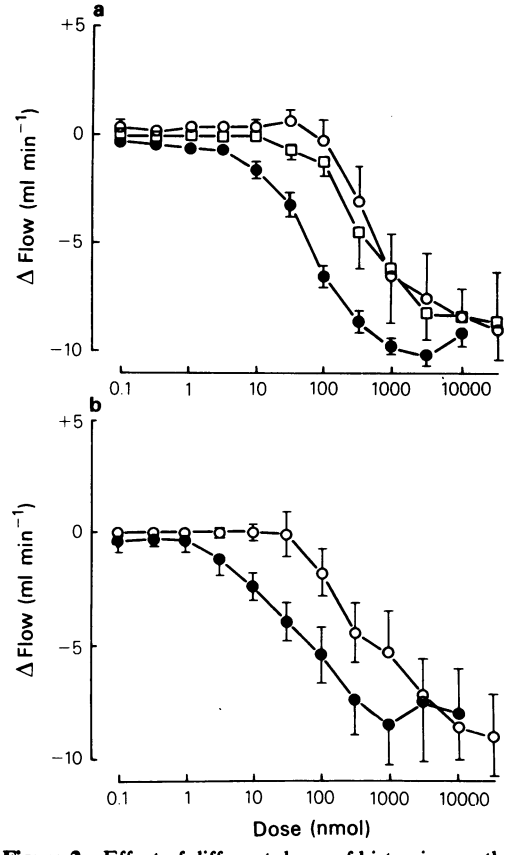

Figure 2 Effect of different doses of histamine on the resistance of the cystic duct of pentobarbitone anaesthetized dogs under control conditions (●) and following treatment with (a) diphenhydramine (○) or cimetidine (□) or (b) with prazosin (○). Histamine was injected in a retrograde manner through the gastroduodenal artery into the hepatic artery and antagonists were given intravenously. Changes in the flow of saline, perfused at constant pressure, were taken to reflect changes in cystic duct resistance. Points represent the mean for curves obtained from 6–8 dogs; vertical lines indicate s.e.mean.

Results

Cystic duct resistance in vivo

Histamine (0.1 to 3000 nmol, i.a.) caused dose-related increases in cystic duct resistance (Figure 2). Responses were rapid in onset (usually within 20 s); recovery varied from 30 s with low doses to 3–4 min with higher doses. As expected, histamine stimulated duodenal motility. However, changes in arterial pressure were evident only with the higher doses and these never preceded changes in cystic duct resistance.

Following treatment with the H_1-receptor antagonist, diphenhydramine (2 mg/kg, i.v.), the increase in cystic duct resistance produced by histamine (Figure 2) was antagonized and slight decreases in cystic duct resistance became apparent at the lower doses of histamine (0.1 to 30 nmol, i.a.). Diphenhydramine also antagonized the effects of histamine on duodenal motility and arterial pressure (data not shown). Histamine-mediated increases in cystic duct resistance were also antagonized by the H_2-receptor antagonist, cimetidine (10 mg/kg i.v.) or by the α-adrenoceptor antagonist, prazosin (1 mg/kg i.v.). In contrast to diphenhydramine, no histamine-mediated decreases in resistance were observed with these antagonists (Figure 2).

The H_1-receptor agonist, 2-pyridylethylamine (PEA, 0.05 to 15 μmol, i.a.) also produced dose-dependent increases in cystic duct resistance (Figures 3 and 5) whereas the H_2-receptor agonist, 4-methylhistamine (MH, 0.05 to 15 μmol, i.a.) produced dose-dependent decreases (Figures 4 and 5) in cystic duct resistance (Figure 2). As has been reported previously (Durant, Ganellin & Parsons, 1974), the so called 'selective' agonists were less potent than histamine. These effects were antagonized following treatment with the H_2-receptor antagonist, cimetidine (10 mg/kg i.v.), and the H_1-

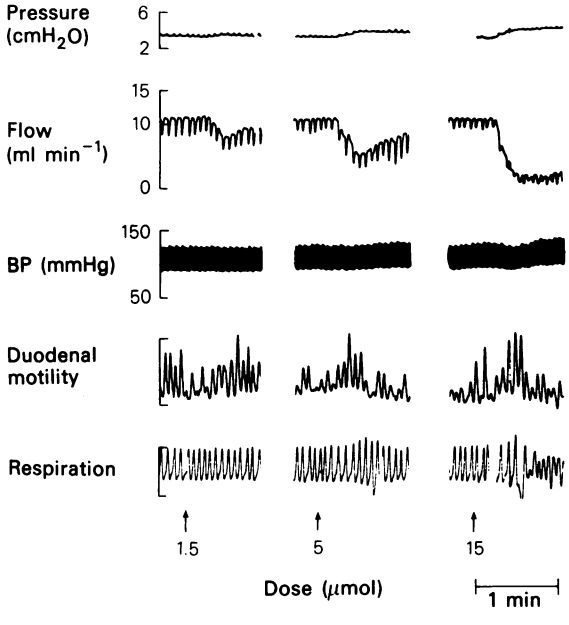

Figure 3 Portions of a typical recording of the effects of the administration of several doses of the H_1-receptor agonist, 2-pyridylethylamine (PEA), into the hepatic artery of anaesthetized dogs. Traces represent (top to bottom) cystic duct perfusion pressure (cmH_2O), flow of saline through the cystic duct (ml min^{-1}), arterial pressure (mmHg), duodenal motility and respiratory movements (arbitrary scale). PEA produced dose-dependent decreases in the flow of saline through the cystic duct. The spontaneous oscillations in flow can be correlated with respiratory movements.

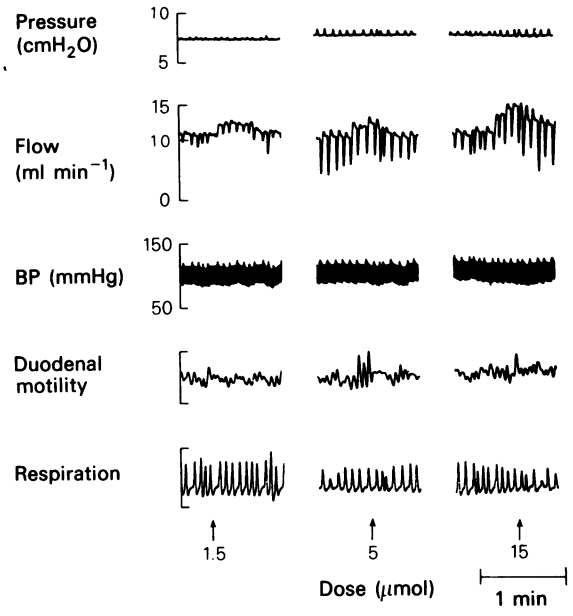

Figure 4 Portions of a typical recording of the effects of the administration of several doses of the H_2 receptor agonist, 4-methylhistamine (MH), into the hepatic artery of anaesthetized dogs. Traces same as for Figure 3. MH produced dose-dependent increases in cystic duct flow indicating a decrease in cystic duct resistance.

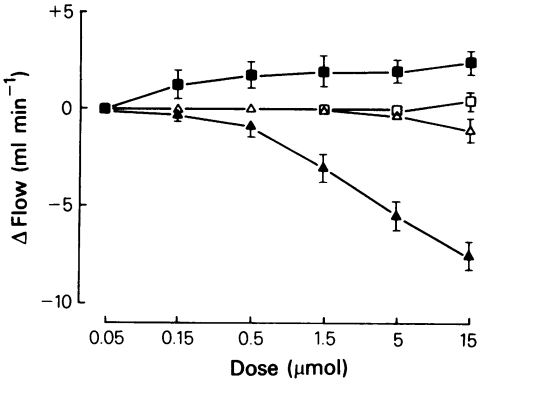

Figure 5 Effect of cimetidine (□) or diphenhydramine (△) on the changes in cystic duct flow (ml min^{-1}) produced by different doses of 2-pyridylethylamine (▲) or 4-methylhistamine (■), respectively, in dogs anaesthetized with pentobarbitone. Points represent the mean for experiments performed in 6 animals; vertical lines indicate s.e.mean.

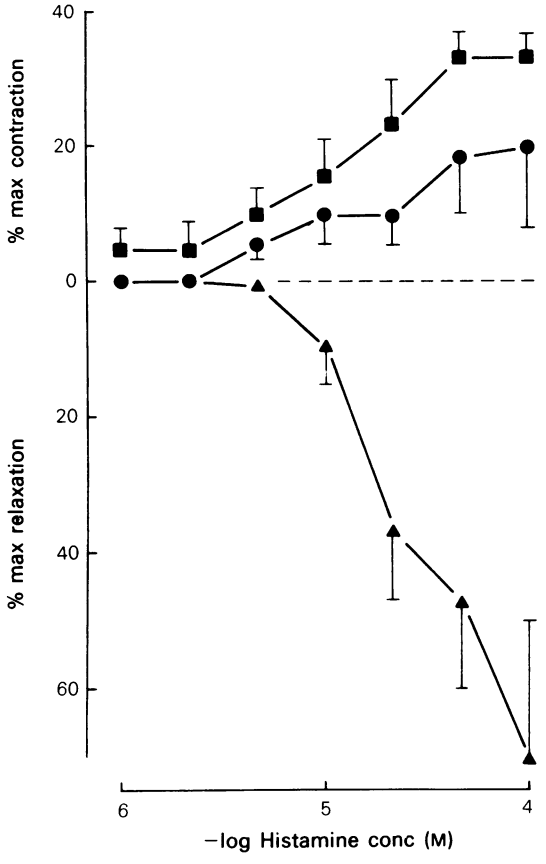

Figure 6 Effect of cimetidine (10 μM, ■) or diphenhydramine (1 μM, ▲) on histamine-mediated (●) contractions of isolated strips of canine cystic duct. Contractile and relaxant responses are expressed as a percentage of the maximum response obtained to supramaximal concentrations of acetylcholine and isoprenaline, respectively. Points represent the mean of $n = 8$; vertical lines indicate s.e.mean.

receptor antagonist, diphenhydramine (2 mg/kg, i.v.), respectively (Figure 5). These doses of antagonists had no direct effects on cystic duct resistance.

Cystic duct contractility in vitro

Under the experimental conditions employed, the cystic duct displayed no spontaneous oscillations in tone. Histamine (1 to 100 μM) produced concentration-dependent contractions of cystic duct (Figure 6). These responses were small compared to responses elicited by ACh; the maximum response to histamine was approximately 20% of that obtained to ACh. These responses to histamine seemed to be the result of stimulation of both H_1- and H_2-histamine receptors as the H_2-receptor antagonist, cimetidine (10 μM), significantly potentiated the contractile response of histamine, whereas the H_1-receptor antagonist, diphenhydramine (1 μM), transformed the effect of histamine from stimulatory to inhibitory. The muscarinic antagonist, atropine (0.1 μM), had no effect on the action of histamine. Following H_1-receptor blockade, histamine produced concentration-dependent inhibitory responses (Figure 6) presumably via an action at H_2-receptors. These inhibitory responses were evident in the absence of drug-induced smooth muscle tone. That stimulation of H_1- and H_2-receptors produced stimulatory and inhibitory responses, respectively, was confirmed by the demonstration that the H_1-receptor agonist, PEA (1 to 100 μM) contracted the cystic duct strips, whereas the H_2-receptor agonist, MH (1 to 100 μM) relaxed the muscle (Figure 7). The specificity of these effects was shown by the antagon-

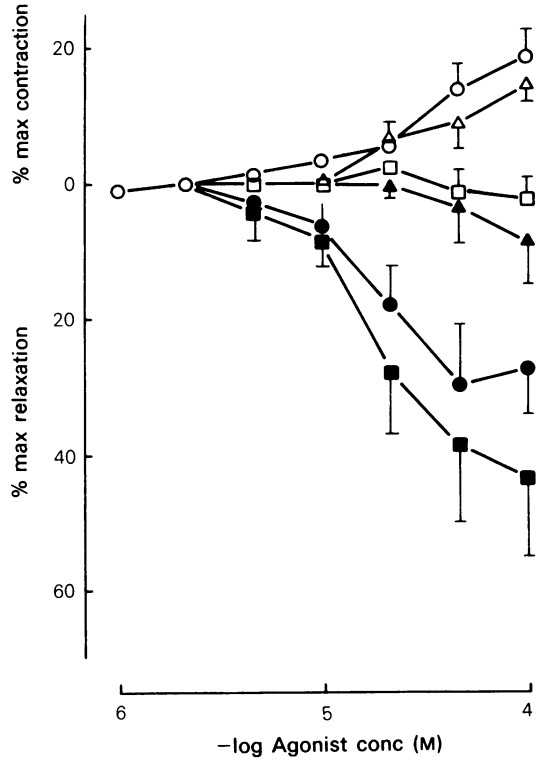

Figure 7 Effect of diphenhydramine (1 μM, ■, □) or cimetidine (10 μM, ▲, △) on the responses of isolated strips of canine cystic duct to 4-methylhistamine (closed symbols) and 2-pyridylethylamine (open symbols). Contractile and relaxant responses are expressed as a percentage of the maximum response obtained to supramaximal concentrations of acetylcholine and isoprenaline, respectively. Points represent the mean of $n = 6$; vertical lines indicate s.e.mean.

ism of the effects of PEA and MH by diphenhydramine and cimetidine, respectively, and lack of antagonism of PEA and MH by cimetidine and diphenhydramine, respectively (Figure 7).

Discussion

H_1- and H_2-receptor agonists and antagonists have been widely used to map the distribution of the two receptors in mammalian tissues. Our findings indicate that both H_1- and H_2-histamine receptors are present in the canine cystic duct, stimulation of which can influence cystic duct resistance. Both PEA (H_1-agonist) and MH (H_2-agonist) are relatively specific for their respective receptors (Durant, Ganellin & Parsons, 1974). PEA increases cystic duct resistance *in vivo* and contracts cystic duct strips *in vitro* which indicates that H_1-receptors are stimulatory. H_2-receptors appear to be inhibitory as suggested by the observation that MH decreases cystic duct resistance *in vivo*, and relaxes cystic duct strips *in vitro*. The predominant effect of histamine on the canine cystic duct is H_1-receptor-mediated stimulation as evidenced by diphenhydramine-sensitive increases in resitance *in vivo* and contractions *in vitro*. However, histamine is probably also exerting some effect via H_2-receptors as cimetidine enhances its contractile effects *in vitro*. Following blockade of H_1-receptors, histamine is capable of eliciting effects via stimulation of H_2-receptors; it could then decrease resistance *in vivo* and relax strips *in vitro*. A second component to the histamine-mediated increase in cystic duct resistance was observed *in vivo* which was sensitive to blockade by cimetidine. This was an unexpected finding as the results obtained using isolated cystic duct strips would suggest that cimetidine should have potentiated the histamine-induced increase in cystic duct resistance *in vivo*. The mechanism of this effect remains unclear. It cannot be explained by the presence of stimulatory H_2-receptors as the H_2-receptor agonist, MH, produced only cimetidine-sensitive inhibitory responses, both *in vitro* and *in vivo*. An α-adrenoceptor component is also indicated as prazosin inhibited the stimulatory effects of histamine *in vivo*, but not *in vitro*. Consequently, histamine may have induced a facilitation or a reflex activation of noradrenergic nerves or a stimulation of catecholamine release from the adrenal medullae (Burn & Dale, 1926; Staszewska-Barczak & Vane, 1965) which produced an α-adrenoceptor-mediated stimulation of the duct.

Stimulatory H_1- and inhibitory H_2-receptors have been identified in vascular smooth muscle (Wood & Simkins, 1973; Ercan, Bokesasy & Turker, 1974), in the pulmonary vasculature in dogs (Turker, 1973), in oesophageal smooth muscle (Cohen & Snape, 1975; deCarle, Brody & Christensen, 1976) and more recently in the gallbladder (Waldman *et al.*, 1977; Gadacz, 1978; Impicciatore, 1978).

Histamine exerts significant effects on gallbladder and, as we have shown, also on cystic duct. However, the role of histamine-mediated responses in the extrahepatic biliary system remains uncertain. The paradoxical occurrence of contraction of both gallbladder and cystic duct smooth muscle in response to histamine may be analogous to our similar finding in dogs *in vivo* (Courtney, Clanachan & Scott, unpublished) that both these tissues were stimulated by CCK. Although CCK caused dose-related increases in gallbladder intraluminal pressure, it also caused dose-related increases in cystic duct resistance. Such an increase in resistance would act to prevent the normal process of gallbladder evacuation. However, comparison of the sensitivities of the gallbladder with cystic duct *in vivo* and *in vitro* revealed that the gallbladder displayed the greater sensitivity to CCK, suggesting that contraction of the gallbladder is not necessarily accompanied by an increase in cystic duct resistance. A similar difference in sensitivity of gallbladder and cystic duct to histamine probably also exists.

The presence of histamine receptors in the cystic duct is of clinical interest. Histamine is probably released locally as part of the inflammatory reaction which accompanies gallstone disease. The inflammatory reaction with acute cholecystitis is usually very marked in the region of the cystic duct. Secondly, patients with duodenal ulceration are now very commonly treated with H_2-receptor antagonists to reduce gastric acid secretion. This therapy might produce constriction of the cystic duct, but this possibility has not yet been investigated.

This work was supported by a grant MA-7336 from the MRC (Canada). D.F.C. was a Postdoctoral Research Fellow of the Alberta Heritage Foundation for Medical Research. We thank Ms Dawne Colwell for expert technical assistance.

References

ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac Chemother.*, **27**, 427–439.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385–390.

BURN, J.H. & DALE, H.H. (1926). The vasodilator action of histamine and its physiological significance. *J. Physiol.*, **61**, 185–214.

COHEN, S. & SNAPE, W.J., JR. (1975). Action of metiamide on the lower esophageal sphincter. *Gastroenterology*, **69,** 911–919.

de CARLE, D.J., BRODY, M.J. & CHRISTENSEN, J. (1976). Histamine receptors in esophageal smooth muscle of the opossum. *Gastroenterology*, **70,** 1071–1075.

DOYLE, J.S. & FARRAR, J.T. (1969). A sphincteric mechanism in the cystic duct of dogs. *Irish J. Med. Sci.*, **7,** 2, 109–117.

DURANT, G.J., GANELLIN, D.R. & PARSONS, M.E. (1974). Chemical differentiation of histamine H_1- and H_2-receptor agonists. Abstract no. Medi 29. In *Abstracts of the 168th National Meeting of the American Chemical Society*, Atlantic City, N.J., 1974.

ERCAN, Z.S., BOKESASY, T.A. & TURKER, R.K. (1974). A study of the histamine H_2-receptors in heart muscle and coronary vessels. *Eur. J. Pharmac.*, **27,** 259–262.

GADACZ, T.R. (1978). Effect of an H-2 antagonist on gallbladder contraction. *J. Surg. Res.*, **25,** 334–341.

IMPICCIATORE, M. (1978). Occurrence of H_1- and H_2-histamine receptors in the guinea-pig gallbladder *in situ*. *Br. J. Pharmac.*, **64,** 219–222.

LaMORTE, W.W., GACA, J.M., WISE, W.E., BIRKETT, D.H. & WILLIAMS, L.F. (1980). Choledochal sphincter relaxation in response to histamine in the primate. *J. Surg. Res.*, **28,** 373–378.

POTTER, J.C. & MANN, F.C. (1926). Pressure changes in the biliary tract. *Am. J. med. Sci.*, **171,** 202–217.

SCOTT, G.W. & OTTO, W.J. (1979). Resistance and sphincter-like properties of the cystic duct. *Surg. Gynecol. Obstet.*, **149,** 177–180.

STASZEWSKA-BARCZAK, J. & VANE, J.R. (1965). The release of catecholamines from the adrenal medulla by histamine. *Br. J. Pharmac. Chemother*, **25,** 728–742.

TORSOLI, A., RAMORINO, M.L. & ALESSANDRINI, A. (1970). Motility of the biliary tract. *Rendic. R. Gastroenterol.*, **2,** 67–72.

TURKER, R.K. (1973). Presence of histamine H_2-receptors in the guinea-pig pulmonary vascular bed. *Pharmacology*, **9,** 306–311.

WALDMAN, D.B., ZFASS, A.M. & MAKHLOUF, G.M. (1977). Stimulatory (H_1) and inhibitory (H_2) histamine receptors in gallbladder muscle. *Gastroenterology*, **72,** 932–936.

WOOD, C.J. & SIMKINS, M.A., ED. (1973). *Proceedings of International Symposium on Histamine H_2-Receptor Antagonists*, London, 1 & 2 Oct., 1973. Welwyn Garden City, Smith Kline & French Laboratories.

(*Received May 19, 1982.*
Revised July 9, 1982.)