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Influence of indomethacin on effects of endothelin-1 on guinea pig isolated rings of common bile duct and sphincter of Oddi

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Abstract

The effects of endothelin-1 on motility of guinea pig extra-hepatic biliary tract portions were studied. Endothelin-1 (≤ 100 nM) failed to contract rings of hepatic, cystic, proximal or distal common bile ducts, or choledochal or papillary halves of sphincter of Oddi. At 100 nM, endothelin-1 or sarafotoxin S6c (selective endothelin ET_B receptor agonist) inhibited contractions of choledochal (but not papillary) sphincter of Oddi to carbachol (1 μM) by 63 ± 5 and 45 ± 9%, respectively. In distal common bile duct, indomethacin (5.6 μM) unmasked potent contractile effects of endothelin-1 [EC_{50} 7.8 (5.5–11.1) nM; E_{MAX} 80 ± 6% of response to 80 mM KCl] and enhanced the contractile potency of carbachol (585-fold at EC_{50} level), but not cholecystokinin C-terminal octapeptide. Inhibition of cholinergic responsiveness of the choledochal sphincter of Oddi by endothelin-1 was reduced by BQ-123 (1 μM; endothelin ET_A receptor antagonist; cyclo[DTrp-DAsp-Pro-DVal-Leu]) and abolished by either BQ-123 plus BQ-788 (1 μM; endothelin ET_B receptor antagonist; *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine) or indomethacin. Thus, eicosanoids of the cyclo-oxygenase pathway (i.e. prostanoids) suppress endothelin-1-induced contractions of distal common bile duct and mediate endothelin ET_A and ET_B receptor-dependent inhibition of cholinergic responsiveness of the choledochal portion of the sphincter of Oddi.

Keywords: Hepatobiliary; Endothelin; Eicosanoid; Prostanoid; Oddi sphincter; Common bile duct; Choledocho-duodenal junction

1. Introduction

Peptides of the endothelin family, comprising endothelin-1, endothelin-2 and endothelin-3 (each with 21 residues), as well as endothelin-1-(1–31) and endothelin-1–(1–32) (Inoue et al., 1989; Wypij et al., 1992; Fernandez-Patron et al., 1999), cause potent and widespread biological effects via activation of at least two specific G protein-coupled receptors, named endothelin ET_A and ET_B receptors (for review, see Kedzierski and Yanagisawa, 2001). Both receptor types can stimulate multiple and complex intracellular signalling mechanisms, including activation of phosphoinositide hydrolysis and mitogen-activated protein kinase and modulation of adenylate cyclase, to mention a few (for review, see Sokolovsky, 1995). In many tissues and organs, effects mediated through endothelin ET_A and/or ET_B receptors depend on or are modulated by eicosanoids of the cyclo-oxygenase pathway (i.e. prostanoids; for reviews, see Hyslop and De Nucci, 1992; Rae et al., 1995). This is due to coupling of these receptors to phospholipase A_2 activation, either directly (Resink et al., 1989) or via protein kinase C, activated through the phospholipase C and D pathways (Wright and Malik, 1996), and/or mitogen-activated protein kinase (Husain and Abdel-Latif, 1999). Moreover, in some cells, endothelin-1 can also upregulate expression of phospholipase A_2 and cyclo-oxygenase 1, but not cyclo-oxygenase 2 (Schramek et al., 1994; Gallois et al., 1998).

In the hepatobiliary system, endothelin-1 is synthesised by stellate cells (also known as Ito cells or lipocytes; Pinzani et al., 1996), hepatic endothelial cells (Eakes et al., 1997) and hepatocytes (Kuddus et al., 2000), as well as by peribiliary mast cells (Koda et al., 2000) and the epithelium lining the intra- and extrahepatic biliary tract (Housset et al., 1993a). Moreover, both endothelin receptors are present in the liver, where the predominance of the ET_A over ET_B type seen in hepatic stellate cells is reversed in sinusoidal endothelial cells (Housset et al., 1993b; Yokomori et al., 2001) and Kupffer cells (Stephenson et al., 1995). Intra-

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hepatic endothelin-1 levels are markedly elevated in liver injury caused by galactosamine or bacterial endotoxin (Ohuchi et al., 1995), in human cirrhotic liver (Pinzani et al., 1996), as well as in cirrhosis induced by bile duct ligation or carbon tetrachloride in animals (Gandhi et al., 1996; Rockey et al., 1998). Enhanced endothelin-1 levels during cirrhosis reflect not only greater synthesis (largely by hepatic stellate cells), but also reduced catabolism of the peptide (Kuddus et al., 2000), and is accompanied by substantial increases in hepatic endothelin ET_A and ET_B receptor density (Gandhi et al., 1996; Yokomori et al., 2001). On the other hand, administration of selective endothelin ET_A and/or dual ET_A/ET_B receptor antagonists reduce the portal hypertension (Reichen et al., 1998) and fibrosis associated with experimentally induced cirrhosis (Rockey and Chung, 1996; Kojima et al., 2000).

The biliary tract plays important physiological roles in the hepatobiliary system, by collecting bile secreted by hepatocytes, modifying its volume and composition and storing it in the gallbladder until delivery to the duodenum in response to feeding (Shaffer, 2000). Endothelin-1 inhibits cyclic AMP-dependent anion secretion in human biliary epithelial cells, which are endowed with both endothelin ET_B and, to a lesser degree, ET_A receptors (Fouassier et al., 1998). These cells also secrete mainly endothelin-1, although they also express mRNA for precursors of endothelin-2 and endothelin-3. Moreover, the dual endothelin ET_A/ET_B receptor antagonist bosentan inhibits taurocholate transport by the hepatocanalicular bile salt export pump (Fattinger et al., 2001). Such actions could be of physiopathological importance, especially considering that bile collected from common bile duct of orthotopic liver transplant patients contains 4-fold higher levels of endothelin, and that even higher levels (10-fold) are found in the gallbladder of patients undergoing cholecystectomy (Kraus et al., 1996).

Storage of bile within the biliary tract and its timely delivery to the duodenum depend on interrelations between the secretory pressure in the initial intrahepatic portions of the tract and the pressure differences between the gallbladder, cystic duct, common bile duct and sphincter of Oddi, which are controlled via neuroendocrine-dependent adjustments in smooth muscle tone (for review, see Shaffer, 2000). In this regard, endothelin-1 has been shown to powerfully contract bile canaliculi formed between rat cultured hepatocytes (Kamimura et al., 1993) and gallbladder strips isolated from guinea pig (Moummi et al., 1992; Battistini et al., 1994; Cardozo et al., 1997), rabbit (Cardozo et al., 2000) and humans (Huang et al., 2001). However, the motor effects of endothelins in other portions of the biliary tract are presently unknown. Thus, the aim of the present study was to assess the motor effects of endothelin-1 in preparations of hepatic, cystic and common bile ducts and sphincter of Oddi isolated from the guinea pig. Moreover, as contractions induced by endothelins in the guinea pig gallbladder are substantially mediated and/or modulated by prostanoids (Cardozo et al., 1997; Nora et al., 2000), we have also evaluated the influence of indomethacin, a non-selective cyclo-oxygenase inhibitor (Vane and Botting, 1997), on the effects of endothelin-1 in these portions of the extrahepatic biliary tract.

2. Materials and methods

2.1. Animals

Experiments were conducted in tissues taken from Hartley guinea pigs of either sex, weighing between 300 and 400 g, housed, for at least 7 days prior to use, in large plastic cages maintained in a temperature (22 ± 2 °C) and illumination (lights on from 06:00 to 18:00 h) controlled environment, with free access to food and water. The protocols employed adhere to the recommendations of the European Community guidelines for use of experimental animals and were approved by Ethics Committee for Animal Use of the Universidade Federal de Santa Catarina.

2.2. Isolated tissue preparations

Under light anaesthesia with ether, the animals were killed by a sharp blow to the head and exsanguination. The extrahepatic biliary tract was quickly removed en bloc, care being taken to avoid damage to the Oddi sphincter embedded in the duodenal wall. After cutting away the gallbladder, the tissue was placed in cold oxygenated Krebs' solution (see composition below), freed from adhering tissues (including the duodenal wall adjoining the Oddi sphincter) and divided to yield ring preparations of the cystic, hepatic and common bile ducts and the sphincter of Oddi. The common bile duct was divided transversally into two portions of equal length, originating a proximal and a distal preparation. Likewise, the sphincter of Oddi was also divided transversally into two parts, halfway down the ampulla, to yield a choledochal and a papillary preparation (the halves closest to the common bile duct and duodenum, respectively). Preparations of cystic and hepatic ducts were not divided. Each preparation was then transferred to a double-jacketed glass organ bath containing 5 ml of Krebs' solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.9, $NaHCO_3$ 25 e glucose 11; pH 7.2–7.4) at 37 °C, continuously bubbled with a mixture of 95% O_2 and 5% CO_2, and connected to force-displacement transducer coupled to a pen recorder, for recording of isometric contractions under basal loads of either 500 (all duct preparations) or 300 mg (both Oddi sphincter preparations).

After equilibration for 1 h (with medium renewals every 15 min), each preparation was always challenged for 10 min with KCl-rich solution (80 mM KCl; obtained by equimolar substitution of NaCl with KCl in the Krebs' solution) as a standard stimulus causing the 100% response, against which all subsequent responses were compared. Following replacement of the KCl-rich solution with normal medium, each

preparation was left to recover for at least another 30 min before any drug additions.

In all types of preparation, a single concentration–response curve was obtained to either endothelin-1 (0.1 to 100 nM), cholecystokinin C-terminal octapeptide (CCK-8; 0.1 to 100 nM) or carbachol (10 nM to 300 μM), in most cases by cumulative additions of increasing agonist concentrations every 1–2 min (or when the effect of the former concentration had peaked). In addition, concentration–response curves to CCK-8 in distal common bile duct and to carbachol in both sphincter of Oddi preparations were also obtained by stepwise non-cumulative additions of single concentrations of these agonists for 1–2 min (each followed by at least three renewals of bathing medium), at 10-min intervals. To assess the contribution of endogenous cyclo-oxygenase-derived eicosanoids (i.e. prostanoids) to the effects of endothelin-1, CCK-8 and carbachol in distal common bile duct and Oddi sphincter preparations, we also obtained curves to these agonists in presence of indomethacin (5.6 μM), a non-selective cyclo-oxygenase blocker (Vane and Botting, 1997) added to the bathing medium 30 min beforehand.

Another set of experiments was performed in both choledochal and papillary Oddi sphincter preparations to analyse the influence of prior exposure to endothelin-1, the selective endothelin ET_B receptor agonist sarafotoxin S6c (Masaki et al., 1994) or CCK-8 on contractions triggered by carbachol. To this effect, after obtaining a control response to a sub-maximally effective concentration of carbachol (at 1 and 3 μM in choledochal and papillary sphincter of Oddi, respectively), which was found to be fully reproducible at 30-min intervals, a single concentration of endothelin-1, sarafotoxin S6c (10, 30 or 100 nM) or CCK-8 (100 nM) was added to the bathing medium for 2 min and a second response to carbachol was elicited in its presence. The influence of indomethacin on the effects of endothelin-1 and CCK-8 were also tested. In other experiments in choledochal preparations of Oddi sphincter only, we assessed the influence of selective endothelin ET_A and ET_B receptor antagonists BQ-123 (cyclo[DTrp-DAsp-Pro-DVal-Leu]) and BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarboyl-D-norleucine), respectively (each at 1 μM; Masaki et al., 1994), either alone or in combination on responses induced by endothelin-1 or sarafotoxin S6c (each at 100 nM). Antagonists were added to the medium 10 min before obtaining the second response to carbachol (i.e. 8 min prior to endothelin-1 or sarafotoxin S6c addition).

2.3. Statistical analysis

Agonist-induced contractions are expressed as mean ± S.E.M., as percentages relative to the response triggered by KCl (80 mM). Potencies of contractile agonists are expressed in terms of an EC_{50} (i.e. the concentration causing 50% of the agonist's maximal response—E_{MAX}), and are presented as the geometric means accompanied by their respective 95% confidence limits. Statistical comparisons were performed by analysis of variance, followed by Student's *t*-test for unpaired samples, and $P < 0.05$ was considered to be significant.

2.4. Drugs and solutions

Physiological salt solutions were prepared with salts of analytical grade in double-distilled deionised water. Endothelin-1 and sarafotoxin S6c were purchased from American

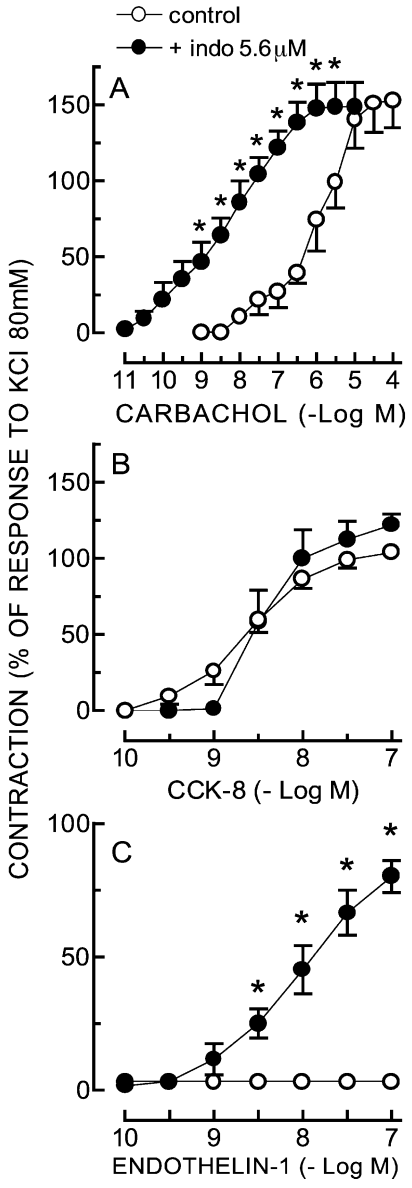

Fig. 1. Responsiveness of rings of distal common bile duct to carbachol (A), CCK-8 (B) and endothelin-1 (C). Cumulative concentration–response curves were obtained in absence (open symbols) or presence of indomethacin (5.6 μM; closed symbols). Values are the mean ± S.E.M. of four to six experiments. * $P < 0.05$ when compared to respective control value (ANOVA and unpaired Student's *t*-test).

Peptide (Sunnyvale, CA, USA); carbachol hydrochloride, [Tyr(SO_3H)^{27}]-cholecystokinin(26–33)-amide (CCK-8) and indomethacin from Sigma (St. Louis, MO, USA); and BQ-788 from Research Biochemicals International (Natick, MA, USA). BQ-123 was synthesized by the Department of Pharmacology, University of Sherbrooke, Canada. Indomethacin was dissolved, immediately prior to use, in absolute ethanol (1 mg ml^{-1}) and then added to the Krebs' solution. Preliminary experiments showed that responsiveness to agonists was unaffected by the final concentration of ethanol achieved in the bathing medium (0.1%; results not shown, $n=2–3$). All other drugs were prepared as stock solutions in phosphate-buffered saline, stored at −18 °C, and diluted to the desired concentrations in the same vehicle just prior to use.

3. Results

3.1. Cystic, hepatic and common bile ducts

Preparations of cystic, hepatic and proximal common bile ducts failed to display spontaneous contractions or motor responses to endothelin-1, CCK-8 (each up to 100 nM), carbachol (up to 100 μM) or KCl (80 mM; results not shown, $n=4$ for each). In contrast, distal common bile duct rings exhibited spontaneous rhythmic contractions and contracted in response to KCl (80 mM) or cumulative additions of carbachol. Although these preparations did not respond to cumulative additions of CCK-8, stepwise non-cumulative additions of this agonist evoked significant concentration-dependent contractions. As depicted in Fig. 1 and Table 1, CCK-8 was about 850-fold more potent (at the EC_{50} level) than carbachol, but induced a far smaller E_{MAX}. Incubation of the distal common bile duct with indomethacin (5.6 μM) unmasked pronounced contractile effects of endothelin-1 and potentiated the responses to carbachol about 585-fold, but did not affect responsiveness to CCK-8.

3.2. Sphincter of Oddi

Both the choledochal and papillary portions of the sphincter of Oddi failed to contract in response to either cumulative or non-cumulative additions of endothelin-1, yet displayed small transient contractions to CCK-8 (only in the non-cumulative protocol at 30 to 100 nM) and graded tonic contractions to carbachol using either protocol. Table 1 summarises the data obtained for these agonists from non-cumulative concentration–response curves only, but responsiveness to cumulative additions of carbachol was very similar to that observed using the non-cumulative protocol [EC_{50} (95% confidence limits) and E_{MAX} (mean ± S.E.M), respectively: choledochal portion 1.62 μM (1.24–2.11) and 132 ± 5%; papillary portion 1.55 μM (1.17–2.05) and 118 ± 5%; $n=4$ to 6 experiments]. In addition to causing tonic contractions, carbachol also increased the frequency of spontaneous phasic contractions in both preparations using either protocol, but this effect was not objectively analysed. Incubation of either Oddi sphincter preparation with indomethacin (5.6 μM) increased the incidence of spontaneous contractions, but did

Table 1
Contractile effects of endothelin-1, CCK-8 and carbachol in rings of guinea pig distal common bile duct (CBD) and choledochal and papillary portions of the sphincter of Oddi (SO), in absence or presence of indomethacin (5.6 μM)

Preparation	Agonist	Condition	EC_{50}[a] (in nM)	E_{MAX}[b]
Distal CBD	Endothelin-1	control	–	no effect
		+ indomethacin	7.8 (5.5–11.1)[c]	80 ± 6[c]
	CCK-8	control	4.5 (2.5–8.2)	104 ± 4
		+ indomethacin	3.7 (2.5–5.6)	122 ± 7
	Carbachol	control	3870 (1720–8510)	156 ± 17
		+ indomethacin	6.6 (3.9–10.9)[c]	148 ± 16
Choledochal SO	Endothelin-1	control	–	no effect
		+ indomethacin	–	no effect
	CCK-8	control	–	small transient effects
		+ indomethacin	–	small transient effects
	Carbachol	control	770 (500–1200)	154 ± 7
		+ indomethacin	810 (430–1540)	181 ± 19
Papillary SO	Endothelin-1	control	–	no effect
		+ indomethacin	–	no effect
	CCK-8	control	–	small transient effects
		+ indomethacin	–	small transient effects
	Carbachol	control	1110 (600–2100)	157 ± 17
		+ indomethacin	1290 (520–3250)	185 ± 18

Data shown concerning responsiveness of CBD or SO were obtained from cumulative or non-cumulative concentration–response curves, respectively.
[a] Geometric means accompanied by 95% confidence limits.
[b] Mean ± S.E.M, expressed as percentage of response to KCl 80 mM.
[c] $P<0.05$ when compared to respective control value (two-tailed unpaired Student's t-test). Each value represents the mean of four to six experiments.

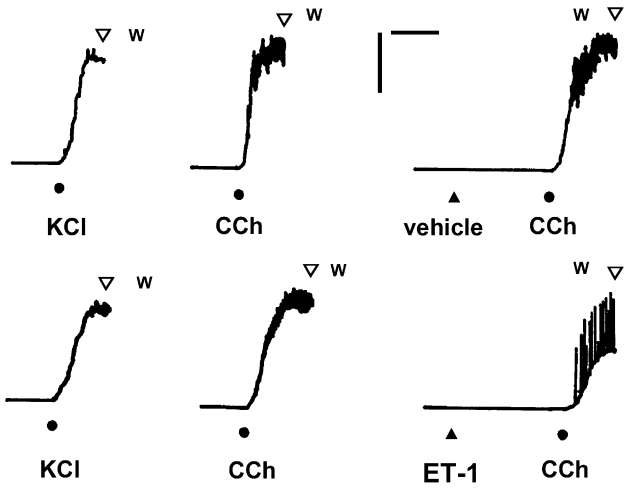

Fig. 2. Representative isometric tracings illustrating the inhibitory effect of endothelin-1 on carbachol-induced contraction of rings of the choledochal portion of the sphincter of Oddi. Preparations were challenged with KCl (80 mM) or carbachol (CCh; 1 μM) for 1–2 min, at 30-min intervals. Endothelin-1 (ET-1; 100 nM) was added to the bath 2 min prior to the second challenge with carbachol (lower trace). Calibration bars indicate 1 min and 0.5 g, respectively, and "W" indicates washout of the agonist. Similar results were obtained in another five experiments.

not significantly affect the responsiveness to non-cumulative additions of carbachol or CCK-8, and failed to uncover any contractile effects of endothelin-1 (Table 1), or sarafotoxin S6c (up to 100 nM; $n \geq 6$).

Repeated challenges of choledochal and papillary Oddi sphincter preparations with 1 or 3 μM of carbachol, respectively, and at 30-min intervals, resulted in reproducible

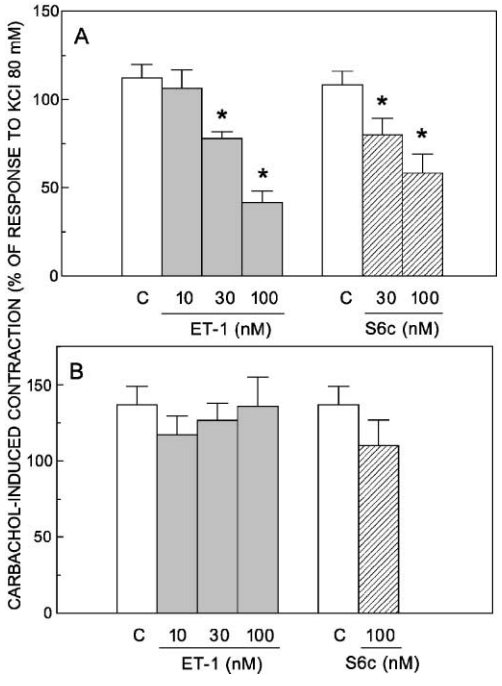

Fig. 3. Influence of endothelin-1 and sarafotoxin S6c on contractions induced by carbachol in rings of either choledochal (A) or papillary (B) portions of the sphincter of Oddi. Preparations were contracted with equieffective concentrations of carbachol (1 μM in A and 3 μM in B) in absence (control; C) or presence of endothelin-1 (ET-1) or sarafotoxin S6c (S6c) at the concentrations indicated. Values are the mean ± S.E.M. of four to six experiments. * $P < 0.05$ when compared to respective control value (ANOVA and unpaired Student's *t*-test).

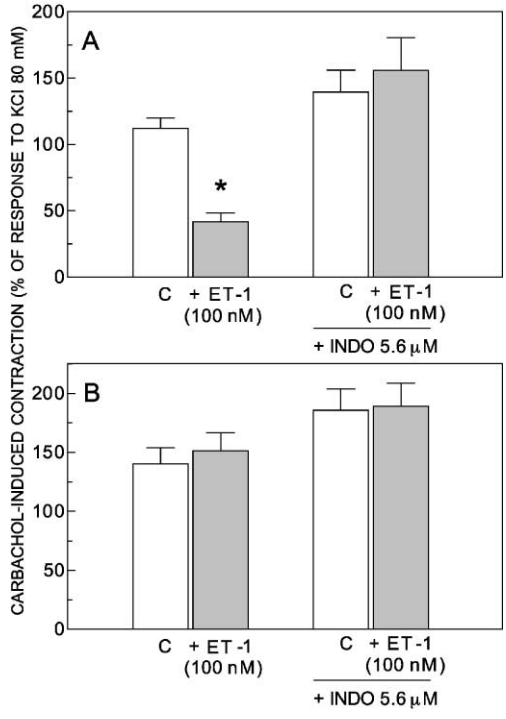

Fig. 4. Influence of indomethacin (INDO; 5.6 μM) on responses induced by carbachol in rings of either choledochal (A) or papillary (B) portions of the sphincter of Oddi, in absence (C; open column) or presence (shaded column) of ET-1 (100 nM). Preparations were contracted with equieffective concentrations of carbachol (1 μM in A and 3 μM in B). Values are the mean ± S.E.M. of six experiments. * $P < 0.05$ when compared to its respective control value (unpaired Student's *t*-test).

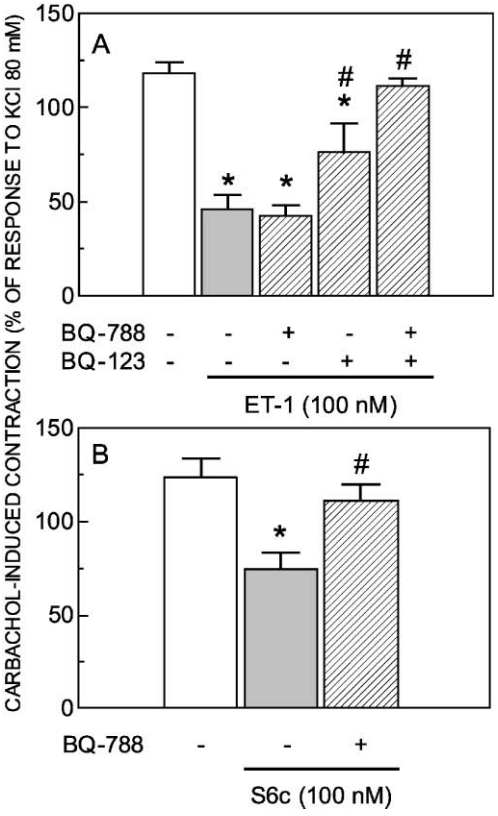

Fig. 5. Influence of BQ-123 and/or BQ-788 (selective endothelin ET_A or ET_B receptor antagonists, respectively; each at 1 μM) on the inhibitory effect of endothelin-1 (ET-1; A) or sarafotoxin S6c (S6c; B) on carbachol-induced contraction of rings of the choledochal portion of the sphincter of Oddi. Values are the mean ± S.E.M. of six experiments. Asterisks and fences denote $P<0.05$ when compared to respective control value (open column) or "agonist without antagonist" value (shaded column), respectively (ANOVA and unpaired Student's *t*-test).

sub-maximal contractions of similar magnitude (choledochal portion 115 ± 4%; papillary portion 125 ± 7%, relative to KCl 80 mM; $n \geq 20$). Addition of endothelin-1 (100 nM) to the bathing medium, 2 min prior to the second challenge with carbachol (1 μM), markedly reduced cholinergic responsiveness of the choledochal Oddi sphincter preparation (Fig. 2). This inhibitory effect of endothelin-1 was clearly concentration-dependent in the 10 to 100 nM range, and was also observed, though to a somewhat smaller extent, when this preparation was exposed previously to the selective endothelin ET_B receptor agonist sarafotoxin S6c (30 and 100 nM; Fig. 3A). At the highest concentrations tested (100 nM), endothelin-1 and sarafotoxin S6c inhibited contractions to carbachol (1 μM) by 63 ± 5% and 45 ± 9%, respectively. In sharp contrast, similar experiments in the papillary portion of the Oddi sphincter failed to detect any influence of either endothelin-1 or sarafotoxin S6c on carbachol-induced responses (Fig. 3B). Moreover, prior addition of CCK-8 (100 nM; 2 min beforehand) to either type of preparation did not affect responsiveness to carbachol (results not shown; $n=6$).

Indomethacin (5.6 μM) fully blocked the depressor effect of endothelin-1 (100 nM) on responsiveness of the choledochal Oddi sphincter to carbachol, without affecting per se the magnitude of contractions induced by the latter (Fig. 4). Also, indomethacin did not affect carbachol-induced contractions of the papillary Oddi sphincter preparation in presence or absence of endothelin-1 (100 nM).

As shown in Fig. 5, the depressor effect of endothelin-1 on choledochal Oddi sphincter responses triggered by carbachol was attenuated by prior incubation with the selective endothelin ET_A receptor antagonist BQ-123 (1 μM), unaffected by the selective endothelin ET_B receptor antagonist BQ-788 (1 μM), but entirely abolished by co-incubation with both antagonists. BQ-788 also abolished the depressor effect of sarafotoxin S6c on carbachol-induced responses of the choledochal portion of the Oddi sphincter. Neither antagonist, alone or in combination, affected carbachol-induced contractions of this preparation (results not shown; $n=4$).

4. Discussion

The results of this study reveal marked differences in the responsiveness of distinct portions of the guinea pig extrahepatic biliary tract to endothelin-1 in vitro, as well as in the involvement of cyclo-oxygenase-derived eicosanoids (i.e. prostanoids) in the mediation of such effects. In this regard, the lack of contractile activity of endothelin-1 in isolated rings of common bile duct, or choledochal or papillary portions of the sphincter of Oddi contrasts with this peptide's remarkable potency in contracting strips of gallbladder (Moummi et al., 1992; Battistini et al., 1994; Cardozo et al., 1997).

We failed to detect any spontaneous or agonist-induced (endothelin-1, carbachol, CCK-8 or even KCl) motor responses of the cystic, hepatic or proximal common bile ducts, even though such portions of the extrabiliary tract contain a sparse population of smooth muscle cells in the guinea pig (Cai and Gabella, 1983). Thus, these portions seem to play only a passive (conductive) role in bile flow to or from the gallbladder in the guinea pig, in contrast to the sphincter-like role played by the smooth muscle present in canine and human cystic duct (Scott and Otto, 1979; Courtney et al., 1983). Indeed, we found that rings of the distal common bile duct, which expresses a far greater density of smooth muscle cells than the proximal common bile duct in the guinea pig (Cai and Gabella, 1983), are spontaneously active and effectively respond to KCl, carbachol and CCK-8, but not endothelin-1. Whole longitudinal preparations of guinea pig common bile duct have also been shown to exhibit contractile responses to metacholine, substance P and other tachykinins (Patacchini et al., 1997). The progressive increase in smooth muscle cell density seen from proximal towards more distal portions of the common bile duct in both guinea pig (Cai and Gabella, 1983) and humans (Hong et al., 2000)

appears to be entirely reversed in the rat, where the responsiveness to either acetylcholine or substance P is highest at the proximal end and decreases towards the choledochal junction (Carrier and Connat, 1995). Such differences could be physiologically relevant, considering the absence of the gallbladder in the rat.

Prior exposure of the guinea pig distal common bile duct to indomethacin clearly unmasked a potent and intense contractile effect of endothelin-1. Thus, the contractile action of endothelin-1 in this preparation is counteracted by prostanoids either present under basal conditions in the tissue, or released in response to the peptide. The first possibility is strengthened by the finding that indomethacin also potentiated carbachol-induced contractions of the distal common bile duct to a remarkable extent (increasing its potency 585-fold). However, the fact that CCK-8-induced contractions were unaffected by indomethacin argues against an indiscriminate inhibitory influence of basal prostanoids on contractility of this portion. In this regard, it seems also noteworthy that CCK-8, unlike bradykinin, fails to trigger prostaglandin E_2 release in rabbit gallbladder, even though both peptides are potent contractile agonists of this preparation (Myers et al., 1992). Furthermore, endothelins are potent and effective releasers of prostanoids in many tissues (for review see Hyslop and De Nucci, 1992), including the guinea pig gallbladder (Cardozo et al., 1997; Nora et al., 2000).

Both choledochal and papillary portions of the Oddi sphincter displayed robust and similar sustained contractions to carbachol, but did not contract in response to endothelin-1 or sarafotoxin S6c (up to 100 nM). Furthermore, in sharp contrast to what was seen in the distal common bile duct, prior incubation with indomethacin failed to affect carbachol-induced contractions or to uncover contractile effects of endothelin-1 in either Oddi sphincter preparation. In fact, in absence of indomethacin, endothelin-1 inhibited carbachol-induced contractions of the choledochal Oddi sphincter substantially, but no effect was seen in the papillary preparation. Prior studies have detected marked differences in the motor and electrophysiological responses of rings of choledochal and papillary Oddi sphincter to transmural electrostimulation (Vongalis et al., 1989; Hirose and Ito, 1991). Thus, the distinct responsiveness of choledochal and papillary preparations of Oddi sphincter to endothelin-1 further substantiates the view that the two regions may exert different functions in choledocho-duodenal junction motility.

Prior incubation of the choledochal Oddi sphincter with indomethacin not only enhanced the incidence and magnitude of spontaneous rhythmic contractions, as previously reported (Gocer et al., 1995), but also abolished the inhibitory effect of endothelin-1 on carbachol-induced contractions. Considering that indomethacin did not alter, per se, contractions elicited by carbachol (or CCK-8), it appears that endothelin-1-induced prostanoid release is a key step in bringing about its depressor effect in this preparation. The identity of the putative prostanoid implicated in endothelin-1-induced inhibition of responses of the choledochal Oddi sphincter to carbachol remains to be clarified. Nevertheless, prostaglandin E_2 appears to be a good candidate for such a role as, unlike prostaglandin $F_{2\alpha}$, it inhibits not only spontaneous Oddi sphincter contractions in the guinea pig, but also contractions triggered by acetylcholine or KCl (Andersson et al., 1976; Gocer et al., 1995). Furthermore, release of prostaglandin E_2 is enhanced in rabbit gallbladder stimulated with bradykinin (Myers et al., 1992) and in guinea pig gallbladder mucosa challenged with capsaicin (Prystowsky and Rege, 1997).

Inhibition of cholinergic (carbachol-induced) responsiveness of the choledochal Oddi sphincter by endothelin-1 appears to be mediated to a substantial extent by endothelin ET_B receptors, as sarafotoxin S6c, a selective agonist of this receptor type (Masaki et al., 1994), also displayed a similar (though slightly smaller) effect. In line with this view, prior incubation of the preparation with BQ-788, a selective endothelin ET_B receptor antagonist, fully prevented the onset of the depressor effect of sarafotoxin S6c. However, the effect of endothelin-1 does not seem to depend solely on activation of endothelin ET_B receptors, as it was also partially prevented by BQ-123, a selective endothelin ET_A receptor antagonist, and fully abrogated by co-incubation of this antagonist together with BQ-788. Although BQ-788 alone failed to affect the inhibitory action of endothelin-1, similar findings have been obtained in other tissues containing a mixed population of endothelin ET_A and ET_B receptors. For example, very high concentrations of either BQ-123 or BQ-788 are completely ineffective in antagonising endothelin-1-induced constriction of rabbit isolated pulmonary artery when applied alone, but fully abolish the response when co-incubated (Fukuroda et al., 1994). Furthermore, we have previously shown that, for reasons which are still unclear, contractions of guinea pig gallbladder induced by endothelin-1 are far less susceptible to inhibition by BQ-123 or BQ-788 than are those triggered by either endothelin-3 or sarafotoxin S6c (in this case only BQ-788 was tested; Cardozo et al., 1997). Altogether, these data demonstrate that the choledochal Oddi sphincter preparation expresses endothelin ET_A and ET_B receptors, both coupled to prostanoid-dependent inhibition of carbachol-induced contractions.

Functional and binding data have established that endothelin ET_A and ET_B receptors also coexist in both the guinea pig and human gallbladder, and seem to be largely restricted to the smooth muscle layer (Cardozo et al., 1997; Huang et al., 2001). In the absence of endothelin ET receptor antagonists, indomethacin diminishes guinea pig gallbladder contractions triggered by endothelin-1, endothelin-3 or sarafotoxin S6c (Nora et al., 2000). Interestingly, however, responses to endothelin-3 mediated by endothelin ET_A or ET_B receptors (i.e. in presence of BQ-788 or BQ-123, respectively) are differentially affected by the cyclo-oxygenase blocker, with ET_B receptor-mediated responses being strong-

ly inhibited and those triggered via ET_A receptors markedly potentiated. Therefore, whereas both endothelin ET_A and ET_B receptors appear to be coupled to mobilisation of depressor prostanoids in the choledochal portion of the Oddi sphincter, in the gallbladder the two receptor types are apparently coupled to generation and release of prostanoids with distinct modulatory influences on smooth muscle contractility.

In conclusion, we have shown that prostanoid-dependent mechanisms counteract the contractile effect of endothelin-1 in the distal common bile duct and effectively mediate the inhibition of cholinergic responsiveness induced by endothelin ET_A and/or ET_B receptor activation in the choledochal, but not the papillary, portion of the sphincter of Oddi of the guinea pig. These actions of endothelin-1 contrast sharply with its pronounced contractile effects in the gallbladder, where endothelin ET_A and ET_B receptors appear to trigger the release of prostanoids with distinct profiles of action (Cardozo et al., 1997; Nora et al., 2000). Epithelial cells lining the human interlobular and common bile ducts and gallbladder mucosa effectively secrete endothelin-1 (Housset et al., 1993a). In this regard, concerted prostanoid-dependent actions of epithelial-derived endothelin-1 in the biliary tract, leading to contraction of the gallbladder and inhibition (relaxation) of distal common bile duct and choledochal Oddi sphincter motility, may constitute an integrated physiological mechanism of bile delivery to the duodenum. Obviously, it remains to be seen if this hypothesis is tenable, especially in view of the complex neuroendocrine mechanisms which control biliary tract motility in vivo.

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