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Vagally mediated cholestatic and choleretic effects of centrally applied Endothelin-1 through ET_A receptors

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Abstract

The role of Endothelin-1 (ET-1) in the central nervous system is not fully understood yet although several studies strongly support its neuromodulatory role. A high density of endothelin receptors is present in the dorsal vagal complex that is the major site for the regulation of the digestive function. Therefore in the present study we sought to establish the role of ET-1 in the central regulation of bile secretion in the rat. Intracerebroventricular ET-1 injection exhibited opposite behaviors on spontaneous bile secretion according to the dose administered. Lower doses of ET-1 (1 fM) increased bile flow and bicarbonate excretion whereas higher doses (1 nM) decreased bile flow and bile acid output. Both the choleretic and the cholestatic effects of ET-1 were abolished in animals pretreated with icv BQ-610 (selective ET_A antagonist) but not with BQ-788 (selective ET_B antagonist). In addition, truncal vagotomy but not adrenergic blockade abolished ET-1 effects on bile secretion. Brain nitric oxide was not involved in ET-1 response since L-NAME pretreatment failed to affect ET-1 actions on the liver. Portal venous pressure was increased by centrally administered ET-1 being the magnitude of the increase similar with low and high doses of the peptide. These results show that centrally applied ET-1 modified different bile flow fractions independent of hemodynamic changes. Lower doses of ET-1 increased bile acid independent flow whereas higher doses decreased bile acid dependent flow. Vagal pathways through the activation of apparently distinct ET_A receptors mediated the cholestatic as well as the choleretic effects induced by ET-1. Present findings show that ET-1 participates in the central regulation of bile secretion in the rat and give further insights into the complexity of brain–liver interaction.

Keywords: Bile flow; Portal venous pressure; Bicarbonate; Bile acids; Glutathione

1. Introduction

Endothelins are a family of related peptides that bind to specific receptors widely expressed in numerous tissues and cell types. The family comprises three isopeptides Endothelin-1 (ET-1), Endothelin-2 (ET-2) and Endothelin-3 (ET-3) that exert different biological effects mainly in an autocrine and/or paracrine fashion [1,2]. ET-1 is produced by the endothelium, brain and gastrointestinal tract and functions as a locally released peptide rather than a circulating hormone. ET-1 plays a relevant role in the regulation of blood pressure either when centrally or peripherally applied acting synergically with other vasoactive substances like angiotensin II and catecholamines [3]. ET-1 also regulates the synthesis and release of various hormones and neurotransmitters [1,4,5].

Two distinct G-protein coupled receptors have been cloned and characterized, ET_A and ET_B [2,6]. The former exhibits a higher affinity for ET-1 and ET-2 than for ET-3 whereas the latter binds the three isopeptides with similar affinity [2]. Alternative splice variants of ET receptors, coupled to distinct intracellular signaling, have been reported but to date their physiological or pathophysiological significance is unclear [2]. A receptor subtype named ETc has been cloned in *Xenopus laevis* and shown to bind specifically ET-3 [7]. Although functional studies support its existence, this receptor has not been cloned in mammals yet [2,5,6]. Endothelin receptors are expressed in several tissues including the endothelium, smooth

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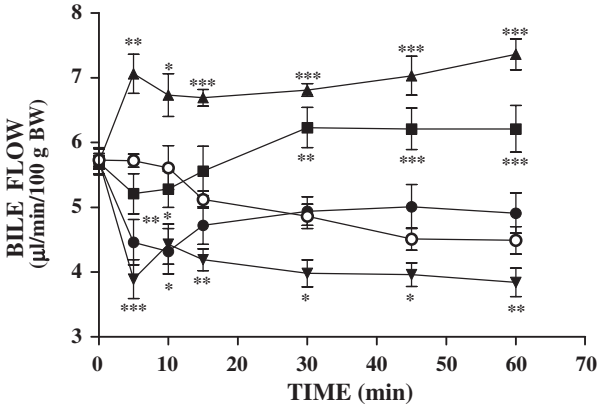

Fig. 1. Effect of centrally applied ET-1 on bile flow (µl/min/100 g BW). ○: Control; ▲: 1 fM ET-1; ■: 1 pM ET-1; ●: 0.1 nM ET-1 and ▼: 1 nM ET-1. *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 10–12. BW: body weight.

muscle, central nervous system (CNS), pancreas and liver among others [2,6].

Although ET-1 and receptors are widely expressed in the CNS, the role of this peptide in the brain is not fully understood. Several studies support its neuromodulatory role and suggest that under physiological conditions it is released mainly from neuronal cells [8,9]. Centrally applied ET-1 acts through ET_A receptors on autonomic neurons to modulate sympathetic outflow [10]. Endothelins and their receptors show specific distribution within the CNS [11]. The central areas where endothelins are highly expressed include the dorsal vagal complex (DVC) formed by the dorsal motor nucleus of the vagus (DMNV) and the nucleus of the solitary tract (NTS), which are the major sites for the autonomic regulation of the gastrointestinal function. Various peptides and neuropeptides influence gastrointestinal motility and/or digestive secretions when applied to the brain. ET-1 through the activation of ET_A receptors acts in the lower brainstem to increase intragastric pressure and gastric smooth muscle contractile activity through vagally mediated pathways [12]. However little is known about the central regulation of bile secretion by peptides as compared with the wide literature on the brain regulation of gastric secretion and gastrointestinal motor function. The secretion of bile is increased by centrally applied neuropeptide Y whereas it is reduced by the icv injection of bombesin or natriuretic peptides [13–15].

In the present work we sought to establish the effect of centrally applied ET-1 on bile secretion and to characterize the receptors and the neural pathways involved. Our findings show that ET-1 applied to the brain evoked opposite dose-dependent regulatory effects on bile secretion through apparently distinct ET_A receptor subtypes via vagally mediated pathways.

2. Materials and methods

Sprague Dawley strain rats (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires) weighing between 250 and 300 g were used in the experiments. The rats were housed in steel cages and maintained at 22–24 °C in a controlled room with 12-h light/dark cycle (light from 7:00 to 19:00 h). All experiments were conducted following the recommendations of the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication N85-23, 1985, revised 1996).

The following drugs were used: ET-1, BQ-610 and BQ-788 (selective ET_A and ET_B receptor antagonist, respectively) (American Peptide Co., Ca, USA); *N*(ω)Nitro L-arginine methyl ester (L-NAME), propranolol, phentolamine and methylene blue (Sigma, St. Louis, MO). Other reagents were of the highest grade available.

One week previous to bile secretion experiments, rats under anesthesia were placed in a stereotaxic instrument (Kopf model

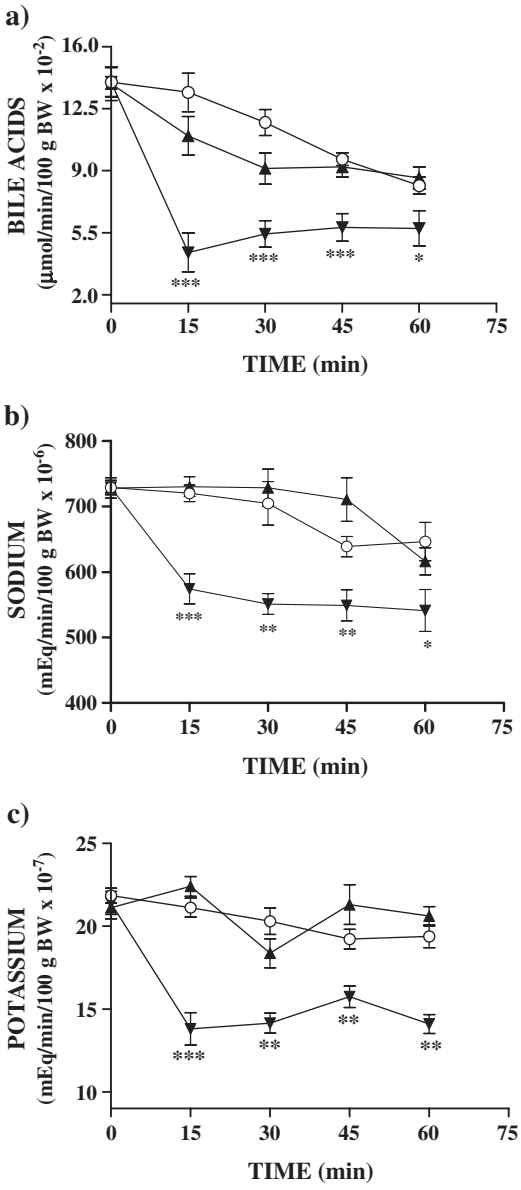

Fig. 2. Effect of centrally applied ET-1 on the output of bile acids (a), sodium (b) and potassium (c). ○: Control; ▲: 1 fM ET-1 and ▼: 1 nM ET-1. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 8–10. BW: body weight.

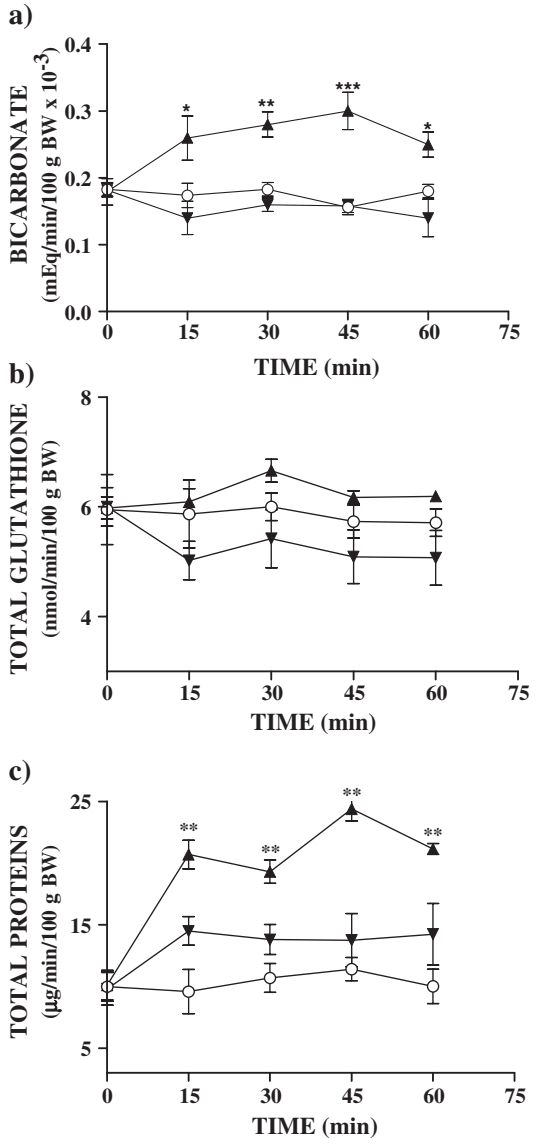

Fig. 3. Effect of centrally applied ET-1 on the excretion rate of bicarbonate (a), total glutathione (b) and proteins (c). ○: Control; ▲: 1 fM ET-1 and ▼: 1 nM ET-1. *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 8–10. BW: body weight.

900, David Kopf Instruments, USA) and an external guide cannula was introduced in the left ventricle of the brain as previously described [13,14]. Briefly, rats were placed on a stereotaxic frame and a small hole was drilled through the appropriate area of the skull, the duramater pierced with a sharp needle, and a 23-gauge stainless steel cannula placed in the left lateral ventricle (1.3 mm posterior to the bregma, 2.0 mm lateral to the midline and 4.0 mm ventral to the skull surface) [16]. The cannula was anchored with a stainless steel screw in the skull to protect the guide cannula and covered by dental acrylic. Rats were placed in individual cages with food (Purina commercial chow) and water ad libitum and allowed 96 h for recovery. For bile secretion experiments rats were fasted for 14 h in order to avoid possible changes in the release of different peptides and/or hormones that may eventually influence bile secretion, and were anesthetized with chloral hydrate (0.4 g/kg, ip). Through a midline abdominal incision the common bile duct was exposed and cannulated with a polyethylene catheter (PC-10 Intramedic, USA) near its bifurcation to avoid contamination with pancreatic juice. In those experiments where drugs were intravenously administered, the left jugular vein was also cannulated with a polyethylene catheter (PC-40 PL, Rivero and Cía., Argentina). Rats remained anesthetized during bile collection that was performed between 9:00 and 11:00 to avoid possible circadian changes [17]. Body temperature was kept at 37 °C with a heating pad.

2.1. Effect of central ET-1 on bile flow and biliary constituents

Bile secretion was allowed to flow for 10 min (basal period) to stabilize the flow. ET-1 (1 fM, 1 pM, 0.1 and 1 nM) (experimental groups) or artificial cerebrospinal fluid (ACSF) (control group) was icv applied at a rate of 1 µl/min (total volume: 2 µl). The ACSF used was of the following composition (mM): NaCl: 125; Ca_2Cl: 1.2; $MgCl_2$: 0.9; $NaCO_3H^-$: 25; $Na_2HPO_4^-$: 0.5; $KH_2PO_4^-$: 0.5; Glucose: 4.3 and urea: 6.5 [13,14]. Bile was collected every 5 min for the first 15 min and every 15 min for 60 min in ice-cold microcentrifuge tubes. The accuracy of icv injections was assessed at the end of each experiment by icv administration of 1 µl methylene blue.

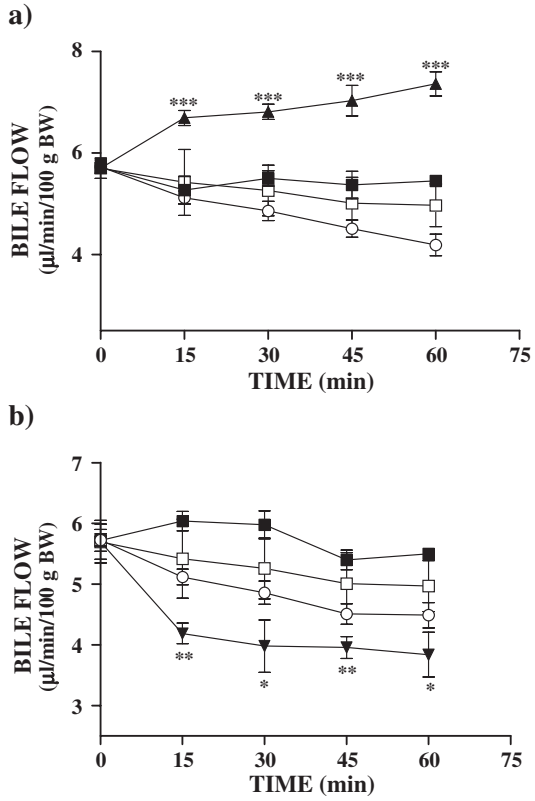

Fig. 4. Effect of ET_A receptor blockade by BQ-610 on 1 fM ET-1 (a) and 1 nM ET-1 (b) induced changes on bile flow. ○: Control; □: 1 µM (a) or 1 mM (b) BQ-610); ▲: 1 fM ET-1; ▼: 1 nM ET-1; ■: 1 µM BQ-610+1 fM ET-1 (a) or 1 mM BQ-610+1 nM ET-1 (b). *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 8–10. BW: body weight.

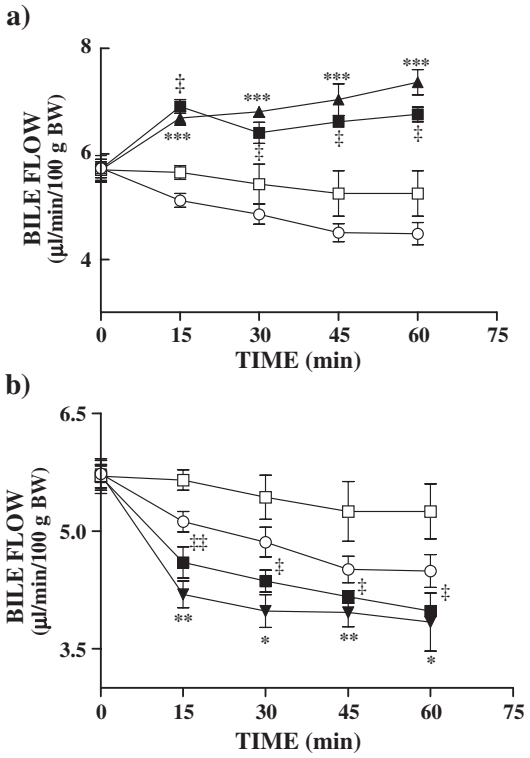

Fig. 5. Effect of central ET_B receptor blockade by BQ-788 on 1 fM ET-1 (a) and 1 nM ET-1 (b) induced changes on bile flow. ○: Control; □: 1 µM (a) or 1 mM (b) BQ-788; ▲: 1 fM ET-1; ▼: 1 nM ET-1; ■: 1 µM BQ-788+1 fM ET-1 or 1 mM BQ-788+1 nM ET-1. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ vs. Control; ‡: $p<0.05$ and ‡‡: $p<0.01$ vs. BQ-788. Number of cases: 8–10. BW: body weight.

Animals were killed and through the opening of the skull the brain was removed, and the presence of methylene blue was strictly verified in the lateral ventricle.

Doses of 1 fM and 1 nM ET-1 were used to determine the receptors and mechanisms involved in ET-1 response on bile secretion.

Biliary sodium, chloride, potassium, and bicarbonate were measured in each sample. The concentration of bile acids and phospholipids in each sample was assessed by the 3α-hydroxysteroid dehydrogenase assay and the modified Bartleet method, respectively [18,19]. The concentration of total glutathione was measured using glutathione reductase and 5,5′-dithiobis 2-nitrobenzoic acid [20]. Proteins were determined according to Lowry et al. [21]. Bile flow was calculated as µl/min/100 g body weight and with these values the excretion rate of the different constituents of bile were determined.

2.2. Endothelin receptors in the brain

To determine the brain endothelin receptor subtype involved in ET-1 response selective ET_A (BQ-610) and ET_B (BQ-788) receptor antagonists were used. BQ-610 (1 µM or 1 mM) and BQ-788 (1 µM or 1 mM) were dissolved in saline and centrally injected 5 min prior to the icv administration of 1 fM or 1 nM ET-1, respectively. Collection of bile samples was performed as described for basal bile secretion experiments.

2.3. Participation of the autonomic nervous system in ET-1 response

When applied to the brain diverse neuropeptides affect gastrointestinal functions through the autonomic nervous system [15]. Therefore we sought to establish whether the parasympathetic or the sympathetic systems were involved in ET-1 response. The role of the vagal pathway was evaluated in animals with bilateral truncal vagotomy. Cervical vagotomy was not performed to avoid possible hemodynamic alterations resulting from the section of the cardiac vagal branches. Bilateral truncal vagotomy was performed by sectioning both branches of the vagus and vagal afferents at the level of the lower esophagus 2 h before bile secretion experiments [13,14,22]. After icv ET-1 (1 fM or 1 nM) bile samples were collected as indicated above.

The contribution of the sympathetic nervous system to ET-1 response was assessed by combined administration of phentolamine (α-adrenergic antagonist) and propranolol (β-adrenergic antagonist). A bolus of 0.5 mg/kg phentolamine was intravenously injected in bolus 30 min before ET-1 central injection. The antagonist was then infused at a constant rate of 0.2 mg/kg/h throughout the period of bile collection. Propranolol (0.5 mg/kg) was intravenously administered 30 min before icv ET-1 injection [13,14,22]. ET-1 (1 fM or 1 nM) was icv injected and

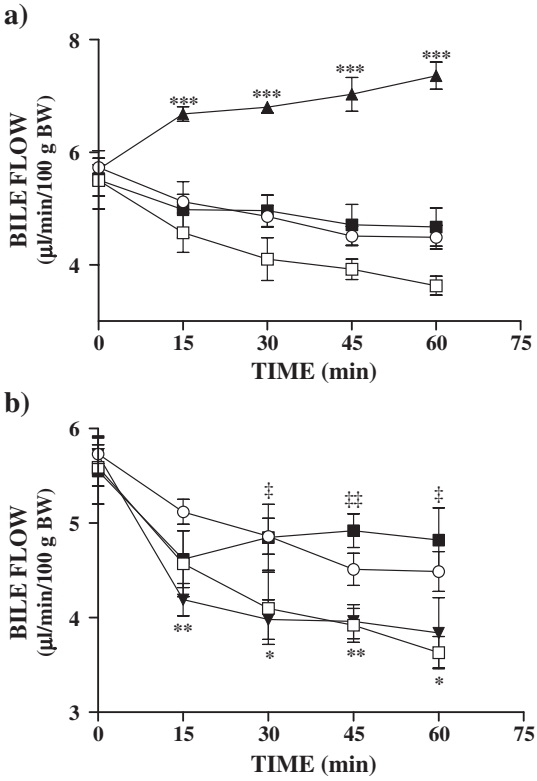

Fig. 6. Effect of centrally applied 1 fM ET-1 (a) and 1 nM ET-1 (b) on bile flow in rats with truncal vagotomy (VT). ○: Control; □: VT; ▲: 1 fM ET-1; ▼: 1 nM ET-1 and ■: VT+1 fM ET-1 (a) or VT+1 nM ET-1 (b). *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control; ‡: $p<0.05$ and ‡‡: $p<0.01$ vs. VT. Number of cases: 8–10. BW: body weight.

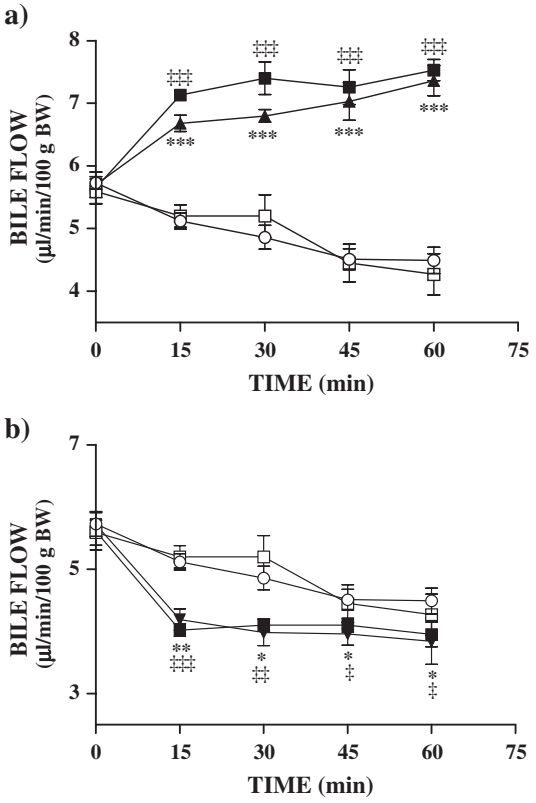

Fig. 7. Effect of adrenergic blockade by phentolamine (P) and propranolol (Pr) on 1 fM ET-1 (a) and 1 nM ET-1 (b) bile flow-induced changes. ○: Control; □: P+Pr; ▲: 1 fM ET-1; ▼: 1 nM ET-1; ■: P+Pr+1 fM ET-1 (a) or P+Pr+1 nM ET-1 (b). *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control; ‡: $p<0.05$; ‡‡: $p<0.01$ and ‡‡‡: $p<0.001$ vs. P+Pr. Number of cases: 8–10. BW: body weight.

samples collected as detailed for spontaneous bile secretion experiments.

2.4. Participation of brain nitric oxide (NO) in ET-1 response

The contribution of NO to ET-1 response was evaluated based on experimental observations showing that NO synthase containing preganglionic neurons are present in the DVC and project to extensive regions of the gastrointestinal tract [23]. To investigate the participation of a central NO pathway in ET-1 response, rats were pretreated with icv 3.8 µM L-NAME 15 min prior to 1 fM or 1 nM ET-1 injection. Bile samples were collected as previously described. The dose of L-NAME used in the present study completely inhibits NO synthase activity in the brain but evokes no hemodynamic changes.

2.5. Portal venous pressure

Portal venous pressure was estimated by splenic pulp pressure as previously described [24]. Briefly, the spleen was exposed by retraction of the perisplenic fat carefully avoiding undue manipulation. A fluid-filled needle end cannula was inserted into the spleen and fixed by applying cyanoacrylate glue. The catheter was connected to a pressure transducer (Staham 923Db) and signals recorded on a polygraph (Coulbourn Inst., USA). After a period of stabilization ET-1 was injected in the lateral ventricle and portal venous pressure recorded. Control rats were injected with the same volume of ACSF (2 µl). Portal venous pressure was monitored during the first 5 min and then every 10 min for 60 min.

2.6. Statistical analysis

The statistical analysis was performed using ANOVA followed by the Student–Newman Keuls test. Portal venous pressure results were analyzed by the Student "t" test. Results are expressed as the mean ± S.E.M. p values of 0.05 or less were considered statistically significant.

3. Results

Results showed that centrally applied ET-1 exhibited opposite effects on bile secretion depending on the given dose (Fig. 1). Lower doses of ET-1 enhanced bile flow whereas higher doses decreased it. One fM ET-1 increased bile secretion at all studied times whereas 1 pM ET-1 induced no changes during the first collection periods (5, 10, 15 and 20 min) but it enhanced bile flow at 30 min up to the end of the experiment.

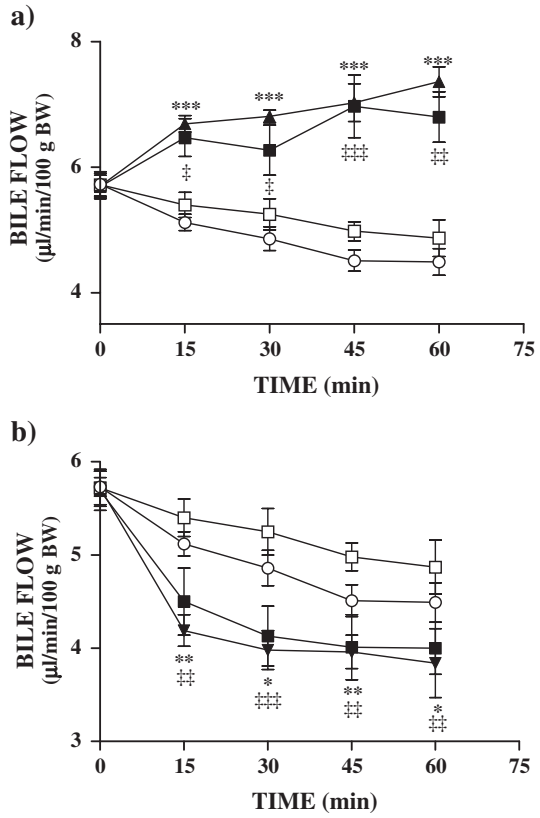

Fig. 8. Effect of NO synthase blockade by L-NAME (3.8 µM) on 1 fM ET-1 (a) and 1 nM ET-1 (b) bile flow-induced changes. ○: Control; □: L-NAME; ▲: 1 fM ET-1; ▼: 1 nM ET-1; ■: L-NAME+1 fM ET-1 (a) or L-NAME+1 nM ET-1 (b). *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control; ‡: $p<0.05$; ‡‡: $p<0.01$ and ‡‡‡: $p<0.001$ vs. L-NAME. Number of cases: 6–8. BW: body weight.

Table 1
Effect of icv ET-1 (1 fM and 1 nM) on portal venous pressure (mmHg)

Time	ET-1 (1 nM) (mmHg)	ET-1 (1 fM) (mmHg)
0 s	4.1±0.7	4.1±0.7
10 s	5.7±0.8*	6.2±0.5**
20 s	6.4±0.1**	5.9±0.6*
30 s	5.8±0.3*	6.2±0.5**
1 min	5.9±0.4*	6.3±0.4**
2 min	6.2±0.2**	6.2±0.3*
3 min	5.8±0.2*	5.9±0.1**
5 min	6.5±0.3**	6.1±0.3**
10 min	5.7±0.2*	5.8±0.3*

*: $p<0.05$ and **: $p<0.01$ vs. time=0. Number of cases: 4–6.

However 0.1 nM ET-1 decreased bile flow but only in the first collection period returning then to control levels whereas 1 nM ET-1 decreased bile flow at all experimental periods. Both the choleretic and cholestatic effects evoked by ET-1 were evident at 5 min after the administration of the peptide. These findings suggest that according to the dose injected ET-1 may trigger different intracellular mechanisms in the brain resulting in either an increase or a decrease in bile flow. Doses of 1 fM and 1 nM ET-1, which increased and decreased bile flow respectively, were used to investigate the neural pathways and receptors involved.

Different biliary constituents were assessed in order to determine the fraction of bile affected by ET-1 applied to the brain. Bile acid output was decreased by 1 nM ET-1 but unaltered by 1 fM ET-1 (Fig. 2a). Further, the excretion of sodium and potassium was also decreased by 1 nM ET-1 but not by 1 fM ET-1 (Fig. 2b and c). On the other hand, chloride excretion was not modified by any dose of ET-1 (data not shown) but bicarbonate output was increased by 1 fM ET-1 and unchanged by 1 nM ET-1 (Fig. 3a). Centrally applied ET-1 modified neither phospholipids (data not shown) nor glutathione excretion at any dose (Fig. 3b). Although 1 nM ET-1 did not affect protein output, 1 fM ET-1 increased it (Fig. 3c).

In order to determine the receptor subtype activated by ET-1 in the brain, experiments were performed in animals pretreated with icv selective endothelin antagonists. Results showed that neither BQ-610 (ET_A antagonist) nor BQ-788 (ET_B antagonist) modified bile flow. BQ-788 did not affect either the cholestatic or the choleretic response of centrally injected ET-1 excluding thus the participation of ET_B receptors (Fig. 4a and b). However both the stimulatory and inhibitory actions of ET-1 were completely abolished in the presence of BQ-610 supporting that both effects were mediated by the activation of central ET_A receptors (Fig. 5a and b).

The participation of the vagal pathway was assessed in rats with bilateral truncal vagotomy. Results showed that truncal vagotomy abolished the effect of 1 fM and 1 nM ET-1 on bile flow supporting that the parasympathetic system mediates central ET-1 response on the liver (Fig. 6a and b). On the other hand, adrenergic blockade by phentolamine and propranolol affected neither basal nor ET-1 induced changes in bile flow (Fig. 7a and b).

The role of brain NO was evaluated by icv L-NAME administration. Blockade of NO synthesis affected neither basal flow nor ET-1 choleretic or cholestatic effects excluding the participation of NO in the central regulation of bile flow by ET-1 (Fig. 8a and b).

Portal venous pressure was significantly increased by icv injection of 1 fM and 1 nM ET-1 and abolished by ET_A blockade as previously shown by other authors (data not shown) [26]. The magnitude of portal pressure elevation was similar with both doses of ET-1 (only results up to 10 min are shown) (Table 1).

4. Discussion

The major finding of the present work was that ET-1 through a vagal-mediated pathway participates in the central modulation of bile secretion by activating apparently distinct ET_A receptors in the brain.

The brain–liver interaction has been widely studied as regards the control of glucose metabolism where the hypothalamus plays a relevant role [25]. However little is known about the existence of a central regulation of bile secretion, although several peptides and neuropeptides have been shown to influence bile flow when they are applied to the brain [13–15].

In the present work centrally applied ET-1 modified bile flow exhibiting opposite effects depending on the given dose. The lowest dose of ET-1 (1 fM) used in the present study increased bile flow whereas the highest dose (1 nM) decreased it. Both the cholestatic and the choleretic effects induced by ET-1 were evident at 5 min after the injection of the peptide. Surprisingly, both the stimulatory as well as the inhibitory effect of ET-1 on bile flow were mediated by the activation of central ET_A receptors. Pretreatment with BQ-610 (selective ET_A antagonist) abolished the stimulatory as well as the inhibitory effect of ET-1 on bile flow, whereas the administration of a selective ET_B antagonist (BQ-788) failed to modify any response. These findings suggest that the activation of ET_A receptors by ET-1 may trigger different intracellular signaling pathways in the CNS according to the dose resulting in either decreased or increased bile flow. Similar findings were observed by other authors but in isolated perfused livers [26]. ET-1 dose-dependently raises portal venous pressure through ET_A receptor activation but at a high dose it causes decreases in bile flow and bile acid excretion whereas at low doses it enhances it despite pressure elevation [26]. These results as well as our findings suggest the existence of distinct ET_A receptors. In fact, binding, pharmacological as well as functional studies support the existence of more than the two conventional receptor subtypes ET_A and ET_B that operate within the nanomolar and picomolar ranges [2]. Distinct ET receptors that possess super high affinity sites (picomolar range) are present in the CNS and peripheral tissues [2]. These receptors share some structural and functional features with the previously identified high affinity receptors (nanomolar range), but nevertheless show some differences in structure as well as in biological activity. Although the ET_B receptor has been more studied regarding this issue, experimental evidence supports that the ET_A receptor also shows high and super high affinity binding sites. In this respect, two functionally distinct ET_A subtypes of binding sites were identified in rat mesangial cells [27]. Moreover characterization

of the ET receptors modulating pituitary hormone secretion indicates the involvement of two ET_A receptor subtypes [28]. Similar findings were observed in the rat atrium, C6-glioma cells and fibroblasts [2]. The activation of the high and super high affinity receptors triggers different intracellular signaling. In cerebral slices ET-1 induces phospholipase C activity at concentrations higher than 500 pM but not at lower concentrations suggesting the operation of different receptors [2,29]. Those of the nanomolar sites are coupled to the phosphoinositide hydrolysis pathway whereas those of the picomolar sites were found to be involved in signal transduction via cAMP and cGMP pathways [30–32]. Whether these receptors are identical to the conventional receptors, having similar biological properties and the ability to oscillate between two affinity states (picomolar and nanomolar sites) or whether they represent distinct receptor subtypes remains to be elucidated [2]. In the present work ET-1 applied to the brain evoked opposing actions on bile flow depending on the dose suggesting the activation of distinct ET_A receptor subtypes within the brain.

Diverse peptides applied to the brain mediate their effect through the autonomic nervous system [15]. Vagal stimulation tends to increase bile flow whereas adrenergic stimulation reduces it [33]. In view of the observation that ET-1 exerted a choleretic or cholestatic effect depending on the dose, the contribution of the autonomic nervous system to ET-1 response was investigated. The participation of a vagal pathway was evaluated in rats with bilateral truncal vagotomy. Vagotomy by itself evoked no changes in bile flow although a reducing trend was observed compared with the control group but it abolished both the stimulatory as well as the inhibitory effect of ET-1 on bile secretion. These findings support that the parasympathetic system mediates the opposing actions of ET-1 on bile flow and further suggest that the DVC is likely a target site of action for this peptide. In fact, autoradiographic studies have documented the presence of ET-1 binding sites in the DVC [34]. The DMNV of the medulla oblongata is a major site of origin of vagal preganglionic fibers that control the gastrointestinal system. This nucleus together with the NTS (the major visceral sensory relay cell group in the brain) is often referred to as the DVC. Throughout the brain ET-1 displays distinct binding densities, immunoreactivity and messenger RNA. When injected into a lateral cerebral ventricle ET-1 evokes a remarkably discrete pattern of c-*fos* expression in the brainstem, where the expression is most marked in the NTS and the DMNV [11]. The pattern of c-*fos* expression provides direct cellular evidence for ET's sites of action in the CNS. The ET_A receptor is responsible for c-*fos* induction indicating that ET-1 derived c-*fos* induction is receptor-mediated [11]. These findings support that ET-1 evoked response on bile secretion is likely mediated by modifications in the neuronal activity of the DVC triggered by ET_A receptor activation. At a high dose ET-1 would decrease vagal activity whereas at a low dose it would increase it. Other ET-1 effects on the gastrointestinal tract mediated by the vagus and central ET_A receptors have also been reported [12].

Although ET-1 action on the liver was vagally mediated, we also investigated if there was any contribution by the sympathetic nervous system based on the evidences showing that ET-1 applied to the brain modulates sympathetic outflow and that it also modifies norepinephrine release in the hypothalamus and other brain areas [4,5,10]. In addition, the observation that 1 nM ET-1 reduced bile flow suggested an increased sympathetic outflow to the liver since enhanced sympathetic activity is associated with reduced bile flow [33]. Blockade of α- and β-adrenoceptors failed to affect basal or ET-1 responses on bile secretion supporting that ET-1-evoked sympathetic outflow does not contribute to changes in bile flow, although it mediates peripheral vascular effects as previously reported [10].

NO is synthesized in neurons and acts as a classical neurotransmitter or neuromodulator to influence a variety of physiological functions including those of the digestive system [23]. NOS containing preganglionic neurons are present in the DVC and project to extensive regions of the gastrointestinal tract [23]. Therefore we evaluated whether brain NO was involved in ET-1 choleretic or cholestatic response. Pretreatment with icv L-NAME failed to affect basal flow and ET-1 evoked changes in bile excluding the contribution of brain NO.

We next evaluated the fraction of bile flow affected by icv ET-1. Bile flow is conceptually divided into two fractions, bile acid dependent flow (BADF) which depends upon the canalicular excretion of bile acids and the bile acid independent flow (BAIF) whose origin is still controversial [35]. Various studies support that bicarbonate excretion accounts for BAIF although the output of glutathione and its derivatives have been also shown to play a major role. The contribution of ductal secretion to total bile flow is important in many species, but not in rodents [35]. Central administration of 1 nM ET-1 that diminished bile flow, also reduced bile acid output but affected neither bicarbonate nor glutathione excretion. Furthermore ET-1 at this concentration also decreased the excretion rate of sodium and potassium that are two electrolytes associated with BADF. These findings support that the inhibitory effect on bile flow evoked by 1 nM ET-1 resulted from the reduction of BADF. On the other hand, centrally applied 1 fM ET-1 that increased bile flow affected neither bile salt nor sodium and potassium excretions supporting that the stimulatory effect evoked by icv ET-1 on total bile flow did not result from an increase in BADF. Nevertheless 1 fM ET-1 increased bicarbonate excretion without affecting glutathione output. Taken together these findings suggest that the choleretic effect evoked by 1 fM ET-1 resulted from an increase in BAIF. Increases in BAIF only due to an increase in bicarbonate excretion have been previously reported [36]. Surprisingly biliary protein output was enhanced by 1 fM ET-1 although it was not modified by a higher dose of the peptide (1 nM). The relevance of this finding is presently unknown. The excretion of proteins in bile is associated neither with BADF nor BAIF generation [35]. In fact most of the biliary proteins derive from the plasma pool and reach bile by transcellular or paracellular pathways [35]. The observation that ET-1 through the ET_A receptor modified different fractions of bile flow depending on the given dose further supports the participation of distinct ET_A receptor subtypes coupled to different intracellular signaling pathways as previously discussed.

ET-1 is a potent vasoactive peptide involved in the central as well as peripheral regulation of blood pressure [10,37]. In addition, systemic administration of ET-1 dose-dependently increases portal venous pressure whereas the icv injection causes changes in regional blood flows including the portal flow [37]. Therefore we sought to establish whether the vasoactive properties of ET-1 could mediate the changes in bile secretion. Both 1 fM and 1 nM ET-1, that increased and decreased bile flow respectively, evoked a sustained increase in portal venous pressure. The magnitude of the increase was similar for both doses and abolished by ET_A blockade as shown by other authors [26]. Both doses of ET-1 increased portal venous pressure but the higher dose decreased bile flow whereas the lower dose increased it. These findings support that the cholestatic and choleretic effects induced by different doses of ET-1 are independent of hemodynamic changes. In fact, changes in portal venous pressure do not necessarily reflect on bile flow unless these variations are sufficiently marked so as to overcome the hepatic autoregulatory mechanisms that operate within a range to maintain a constant blood flow along the sinusoids. Furthermore, although ET-1 enhances sympathetic outflow to the liver peripherally adrenergic blockade did not prevent ET-1 response on bile secretion.

Previous reports show that in the isolated perfused liver ET-1 also exerts cholestatic or choleretic effects [26,38,39]. The cholestatic effect was ascribed to hemodynamic changes whereas the choleretic action was attributed to a direct effect on the hepatocyte function [26,38]. Most of the literature regarding ET-1 and hepatic function refers to its role in pathophysiological situations [40].

Present results show that the icv administration of ET-1 through a vagal pathway evoked opposing actions on bile flow mediated by the activation of apparently distinct ET_A receptors in the brain. The fraction of bile flow affected by ET-1 was dependent on the dose of the peptide and independent of hemodynamic changes considering that both doses evoked an elevation of portal venous pressure approximately of the same magnitude. The present work further supports the physiological relevance of the action of neuropeptides on brain sites to control gastrointestinal function and reveals new insights into the brain–liver interaction.

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