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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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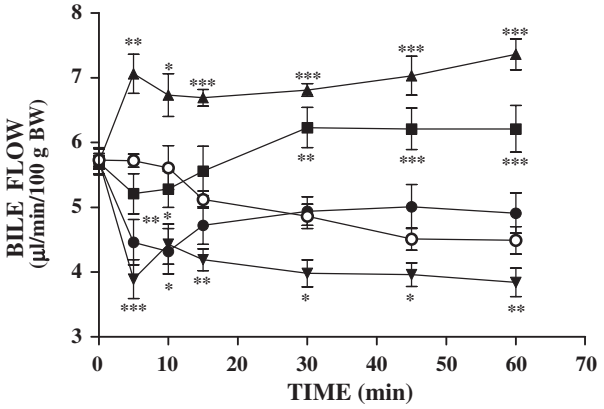

Fig. 1. Effect of centrally applied ET-1 on bile flow (µl/min/100 g BW). ○: Control; ▲: 1 fM ET-1; ■: 1 pM ET-1; ●: 0.1 nM ET-1 and ▼: 1 nM ET-1. *: $p<0.05$; **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 10–12. BW: body weight.

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

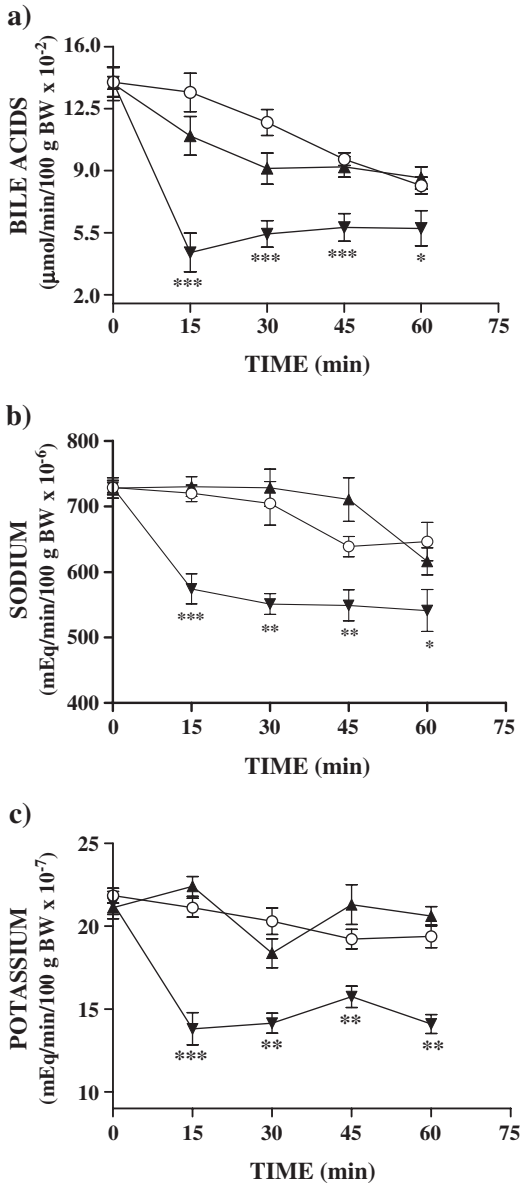

Fig. 2. Effect of centrally applied ET-1 on the output of bile acids (a), sodium (b) and potassium (c). ○: Control; ▲: 1 fM ET-1 and ▼: 1 nM ET-1. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ vs. Control. Number of cases: 8–10. BW: body weight.