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Effect of feeding behavior on circadian regulation of endothelin expression in mouse colon

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ABSTRACT

Aims: The function, regulation and gene expression of the endothelin (ET) system in the intestine is not well understood. We investigated the dependence on feeding schedule and biological clock of the regulation of ET-1 gene expression in mouse colon.
Main methods: Mice were fed freely, fasted for 48 h and re-fed after fasting.
Key findings: Where indicated ET-1 gene expression was highest in the colon compared with other tissues examined in fasted mice. Fasting increased the level, while maintaining the rhythmicity, of ET-1 gene expression in epithelial colonic tissue. Re-feeding, however, decreased ET-1 gene expression and suppressed rhythmic oscillation, and the rhythmicity also changed for gene expression for circadian clocks, period-1 and period-2 (Per1 and Per2). Furthermore, the decrease in ET-1 gene expression induced by re-feeding was blocked by pre-treatment with hexamethonium and atropine. The daily change in ET-1 gene expression in colon, which depends on feeding schedule via the autonomic nervous system, is synchronized with peripheral circadian oscillators under conditions of free feeding and fasting but not re-feeding. The decrease in ET-1 gene expression in the proximal colon induced by re-feeding occurs via the nervous system.
Significance: ET-1 plays an important physiological role, which is dependent on feeding behavior.

Introduction

Endothelin (ET), mediated by two receptors, influences a variety of biological activities in many tissues including vasoconstriction, smooth muscle contraction, cell growth and cell differentiation (Yanagisawa et al., 1988; Pollock, 1998; Andrew and David, 1998; Sakurai et al., 1990; McKay et al., 1991; Wang et al., 2001; Lahav et al., 1996; Baynash et al., 1994). Localization of peptide and gene expression of the three ET isoforms and their receptors in the gastrointestinal tract of mammalian species has been reported (Takahashi et al., 1990; Yoshinaga et al., 1992; Fang et al., 1994; Bloch et al., 1991; Saida et al., 2000). However, the role of the ET system has been studied less extensively in the gastrointestinal tract than in the cardiovascular system.

Studies have suggested physiological and pharmacological actions of the ET endocrine system in the gastrointestinal tract; ET-2/vasoactive intestinal contractor (VIC) was cloned by Saida et al. (1989) and was found to be an intestinal contractor by Ishida et al. (1989). It also has been reported that ET-1 and ET-3 cause a sustained contraction mediated by the ETA and/or ETB receptors in the isolated longitudinal smooth muscle of the guinea pig ileum (Hori et al., 1994) and cecum (Okabe et al., 1995) in vitro. Furthermore, it has been reported that ET-1, ET-2/VIC, and ET-3 contribute to mucosal injury of the stomach in rat and mouse (Morales et al., 1992; Wallace et al., 1989; Kozakai et al., 2002a). In addition, it has been reported that loss of ET-3 and/or ETB receptor function may be involved in the abnormal development of neural crest-derived cells, causing Hirschsprung's disease (Puffenberger et al., 1994; Oue and Puri, 1999; Won et al., 2002). It also has been observed that ET-1 and ET-3 increase chloride secretion

Abbreviations: ET, endothelin; Per, period; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VIC, vasoactive intestinal contractor.

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in rat (Moummi et al., 1992; Gonzalez Bosc et al., 2001) and rabbit (Roden et al., 1992) colon in vitro and that ET-1 inhibits glucose-coupled sodium absorption (Kuhn et al., 1997) and enhances bicarbonate secretion in the small intestine of rat (Takeuchi et al., 1999) in vitro. However the degree to which environmental conditions affect the regulation of ET gene expression in the gastrointestinal tract has not been demonstrated.

Feeding is an important factor influencing the physiologic action of the gastrointestinal tract in many species. To understand the regulation of ET gene expression in the gastrointestinal tract, we studied the effects of feeding and fasting on the gene expression of ET-1 and ET-2/VIC as well as their regulatory mechanisms (including the neuronal system) in mouse colon.

Materials and methods

Animal, tissue and RNA

Male ICR mice obtained from Nippon Clea (Tokyo, Japan) were housed in a temperature-controlled animal room. Lights were kept on between 0700 h and 1900 h each day to maintain 12-hour light and dark periods over a 24-hour cycle. All animals were allowed access to water and food ad libitum and were maintained under these conditions for two weeks. In the re-feeding experiments, food was removed at 1000 h for fasting and was given again at 1000 h after 48 h of fasting. The re-feeding period lasted at least 24 h. In experiments involving intravenous injection, we used the following solutions: 100 μL of hexamethonium bromide solution (a ganglionic inhibitor, 10 μg/g of body weight), atropine solution (a muscarinic inhibitor, 2 μg/g of body weight), a glucose solution (1 mg/g of body weight) and saline as a control. We used 500 μL of glucose solution (2.5 mg/g of body weight) for intragastric injection. Our experimental procedures were in accordance with the Guidelines on Handling of Laboratory Animals for our institution.

Mice (7–8 weeks old) were sacrificed by cervical dislocation. Excised tongue, stomach, duodenum, jejunum, proximal colon and pancreas were washed with ice-cold PBS. These tissues were used for gene expression analysis. Total RNAs were prepared from excised tissues using a commercial kit (Isogen solution; Nippon Gene, Japan).

Real-time polymerase chain reaction (PCR) for ET-1 and ET-2/VIC

The method used has been described previously (Kozakai et al., 2002b). Sample cDNA for real-time PCR was obtained by reverse transcriptase reaction of total RNA. Amplification was carried out by two-step PCR using the TaqMan PCR kit (PE Applied Biosystems, USA). Oligonucleotide primers for murine ET-1 (GenBank accession no. D43775) were 5′-TTCCCGTGATCTTCTCTCTGCT-3′ (sense) and 5′-TCTGCTTGGC-AGAAATTCCA-3′ (antisense). Oligonucleotide primers for murine ET-2/VIC (GenBank accession no. NM_007902) were 5′-CTGCGTTTTCGTCGTTGCT-3′ (sense) and 5′-TGCAGCTCATGGTGTTAT CTCTTC-3′ (antisense). Oligonucleotide primers for murine GAPDH (GenBank accession no. BC096440) were 5′-CTTCACCACCATGGAGAA GGC-3′ (sense) and 5′-GGCAT-GGACTGTGGTCATGAG-3′ (antisense). The detection probes (TaqMan Probe, PE Applied Biosystems, USA) for murine ET-1, ET-2/VIC and GAPDH were FAM-ACAAGGAGTGTG TCTACTTCTGCCAC-CTGG-TAMRA, FAM-CTGCAACTCCTGGCTTGACAAG GAA-TAMRA and FAM-CCTGGCCAAGGTCATCCATGACAACTTT-TAMRA, respectively. Reaction conditions were 95 °C for 10 min followed by 50 cycles of the amplification step (95 °C for 20 s and 62 °C for 2 min). The amplification products from mRNAs were predicted to be 370, 422 and 238 base pairs (bp) for ET-1, ET-2/VIC and GAPDH, respectively. The gene expression rate was obtained by normalizing the amount of ET-1 or ET-2/VIC with that of GAPDH using the following formula:

[amount of ET-1 or ET-2/VIC cDNA in a sample / amount of GAPDH cDNA in a sample] × 100.

Northern blot analysis for period-1 (Per1) and period-2 (Per2)

The method used was the same as that described by Oishi et al. (2002). Total RNA was separated on a 1% agarose/0.7 M formaldehyde gel. Each lane contained 20 μg of total RNA from one tissue. RNA was transferred to a nylon membrane (GeneScreen Plus, DuPont, USA) by passive capillary transfer and probed with ^{32}P-labeled random primed probes. Probes were hybridized to blots at 55 °C, and the final wash was carried out at 55 °C in 0.1 × SSPE/1% sodium dodecyl sulfate for 40 min. Hybridized blots were imaged and analyzed by a BAS 2000 Bio-Imaging Analyzer (Fuji Photo Film, Tokyo, Japan). Hybridization signal of period-1 (Per1) messenger RNA (mRNA) and period-2 (Per2) mRNA was normalized by the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, as described previously (Oishi et al., 2002; Hanai et al., 2005; Sakamoto et al., 1998). The cDNA hybridization probes were generated from cDNA fragments of rat Per1 (bases: 2358–3114; GenBank accession no. XM_340822), rat Per2 (bases: 1123–1830; GenBank accession no. AB016532) and rat GAPDH (GenBank accession no. X02231), because the sequences of the cDNA fragments are almost the same in rat and mouse.

Statistical analysis

The results are represented as the mean + SEM (mean ± SEM). The mean values were analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple range test, except for experiments involving intravenous injection. For the intravenous injection experiments, Welch's *t*-test was used because variant values were different among all groups (Zer, 1984). P values of less than 0.05 were considered statistically significant.

Results

The effect of fasting and re-feeding on the gene expression of ET-1 and ET-2/VIC

We studied the effect of fasting for 48 h followed by re-feeding on the gene expression of ET-1 and ET-2/VIC in the tongue, stomach, duodenum, jejunum, proximal colon and pancreas of mouse ($n = 3$). Both ET-1 and ET-2/VIC genes were expressed in the tongue, stomach, duodenum, jejunum, proximal colon and pancreas of freely fed mice (Figs. 1 and 2). In these mice the highest ET-1 level was observed in the pancreas (2.23 + 1.24, Fig. 1). The 48-hour fast increased ET-1 gene expression, and this increase was most significant in the proximal colon and duodenum in comparison with the other tissues (proximal colon: 3.86 + 1.1, duodenum: 0.06 + 0.01, $P < 0.05$). Re-feeding for 0.5 or 2.0 h after the 48-hour fast decreased the fast-induced ET-1 gene expression rates to the same levels as those found in freely fed mice in all tissues examined except the pancreas.

In contrast, the highest level of expression for ET-2/VIC was observed in the jejunum (0.64 + 0.08) of freely fed mice. However fasting and re-feeding did not cause significant change in ET-2/VIC gene expression in any of the tissues examined (Fig. 2).

We chose, therefore, to investigate in detail the mechanism by which fasting and re-feeding affect the regulation of ET-1 gene expression. Based on the observation that proximal colon is the site exhibiting the greatest response of ET-1 gene expression to the 48-hour fast, we used tissue from the colon for these additional studies.