British Journal of Pharmacology (2006) 147, 607–611
www.nature.com/bjp

Pharmacological characterization of endothelin receptors-mediated contraction in the mouse isolated proximal and distal colon

[1]Humaira Khan, [1]Robert J. Naylor & *,[1]Bishwa R. Tuladhar

[1]School of Pharmacy, University of Bradford, Richmond Road, Bradford, W. Yorkshire BD7 1DP

1 The study investigated the role of endothelin (ET) and the ET receptor subtypes ET_A and ET_B in mediating longitudinal contraction in the mouse proximal and distal colon.

2 Cumulative concentration–response curves to a range of ET agonists (ET-1, ET-2, ET-3, ($Ala^{1,3,11,13}$) ET and IRL 1620) were established by administering concentrations ranging from 0.01 nM to 0.3 μM. Concentration–response curves to ET-1, which exhibits a high affinity for both ET_A and ET_B receptor subtypes, were also established in the presence of the ET_A antagonist BMS 182874 and the ET_B antagonist IRL1038.

3 The addition of the selective ET_A receptor antagonist BMS 182874 caused a rightward shift of the concentration–response curve to ET-1 in both sections of the colon. The ET_B receptor antagonist IRL1038 (0.3–1 μM) did not significantly effect the response to ET-1 in the proximal colon but caused a significant decrease in response towards higher concentrations ranges ($\geqslant$3 nM) in the distal colon.

4 A comparison of the concentration–response curves to ET-1, ET-2 and ET-3 showed a rank order of potency ET-1$\geqslant$ET-2$\gg$ET-3 in the proximal colon and ET-1$\geqslant$ET-2$\geqslant$ET-3 in the distal colon. The selective ET_B receptor agonists, ($Ala^{1,3,11,13}$) ET and IRL 1620 did not produce any response in the proximal sections of the colon but produced a smaller contraction in the distal segments.

5 The data indicate that ET can contract the proximal tissues of the mouse colon predominantly *via* ET_A receptors and in the distal tissues *via* ET_A and ET_B receptors.
British Journal of Pharmacology (2006) **147,** 607–611. doi:10.1038/sj.bjp.0706657;
published online 23 January 2006

Keywords: Endothelin; mouse proximal and distal colon; endothelin receptors

Abbreviations: EDCF, endothelium-derived contracting factor; ET-1, endothelin-1; ET-2, endothelin-2; ET-3, endothelin-3

Introduction

The initial discovery of the peptidergic endothelium-derived contracting factor (EDCF) began with the pioneering work of Hickey *et al*. (1985) and was confirmed in subsequent studies (Gillespie *et al*., 1986; O'Brian *et al*., 1987). In 1987, Masaki and co-workers isolated, purified, sequenced and cloned this peptide and coined the name 'endothelin' (ET) (Yanagisawa *et al*., 1988; Masaki *et al*., 1994). Subsequent studies have identified three different ET genes in the human genome, which are differentially regulated (Rubanyi & Polokoff, 1994) and express three different isopeptides-ET-1, ET-2 and ET-3 (Inoue *et al*., 1989). Moreover these isopeptides are differentially distributed among several tissues (Kasuya *et al*., 1991) in varied proportions and exert their effects through a number of ET receptor subtypes. Among these the ET_A and ET_B receptors have been molecularly characterized (Sakurai *et al*., 1992), but the existence of a third subtype, ET_C (Karne *et al*., 1993), as a species variant or a new subtype is yet to be ascertained.

ET-binding proteins are widely distributed throughout the gastrointestinal tract, brain, kidneys, lungs, trachea and vascular tissue (Takahashi *et al*., 1990). The gastrointestinal tract of the rat has a specific binding area for ET in the fundus of the stomach, jejenum, ileum and colon, where relatively low concentrations of ET have been shown to elicit a contractile response (Wallace *et al*., 1989; Takahashi *et al*., 1990; Peskar *et al*., 1992).

The mounting interest in ET research, coupled with the discovery of potent agonists and antagonists, may lead to a better understanding of the pathogenic mechanisms in the gastrointestinal tract and the development of novel therapies for gastrointestinal disorders. Moreover, the discovery of potent ET antagonists and the recent demonstration of their effectiveness in animal models of pathology such as Hirschsprung's disease (Zhu *et al*., 2004), has provided preliminary evidence that endogenous ETs may be of importance in the pathogenesis of human diseases (Rubanyi & Polokoff, 1994; Tekin *et al*., 1999; Davenport & Maguire, 2002). The present study investigated the role of ET and its receptors ET_A and ET_B in mediating longitudinal muscle contraction responses in the mouse proximal and distal colon.

Methods

Animals and housing conditions

The experiments were carried out on adult Bantin Kingman White (BKW) mice of either sex weighing between 25 and 35 g. The mice were housed in single sex groups of 10 per cage and allowed food and water *ad libitum*. The polypropylene cages were floored with sawdust and cleaned on a regular basis. The

*Author for correspondence: E-mail: B.R.Tuladhar@bradford.ac.uk

animals were maintained at a humidity of 40–50% at 21°C on a 14/10-h light/dark cycle. Animals were allowed to acclimatize in holding rooms for at least 3 weeks before commencing the experiments.

Tissue preparation

Animals were killed by cervical dislocation and the entire length of the colon, approximately 6–7 cm in length, was removed. The section was kept in Krebs–Heinseleit (KH) (composition mM: NaCl 118, KCl 4.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 10.0) solution oxygenated with 95% O_2 and 5% CO_2. The mesentery and fatty tissue were removed and the luminal contents were carefully washed out with KH solution. The proximal and distal segment of the colon (approximately 2 cm in length and taken 1–2 cm and 4–5 cm, respectively, from the ileo-caecal junction), was dissected and mounted vertically in 10 ml, water-jacketed organ baths containing KH solution kept at 37°C and oxygenated with 95% O_2 and 5% CO_2. Longitudinal contractions were measured isometrically using Grass Force Displacement transducers (FT03C, Grass Instrument Co., MA, U.S.A.) and recorded on a multichannel Powerlab recorder (Powerlab/8SP Data recording system SP8088). The tissues were placed under a resting tension of 1 g and allowed to equilibrate for 30 min with two washouts every 15 min.

Drug administration

Concentration–response curves to ET receptor agonists (0.01 nM–0.3 μM) were established, once a stable baseline tension was obtained, by cumulative administration of the agonist with contact time of 1–2 min for each concentration. In experiments examining the effects of tetrodotoxin (TTX), ET_A and ET_B receptor antagonists on ET-1 responses, the tissues were equilibrated with TTX or the antagonist for at least 30 min before the cumulative concentration–response curves to ET-1 were established.

Drugs

($Ala^{1,3,11,13}$) ET, BMS 182874 (5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide), ET-1, ET-2, ET-3, IRL 1038((Cys11, Cys15) ET-1), IRL 1620 (Suc-(Glu9, Ala11, 15) ET-1 (8–21)) (Tocris) and TTX citrate (Tocris, Bristol, U.K.). All drugs, except TTX which was dissolved in distilled water, were dissolved and diluted in bovine serum albumin (0.01%). Drugs were added directly into the organ baths in volumes not exceeding 1% of the bath volume.

Analysis of results

A difference between two groups was compared using the Student's *t*-test (unpaired, two-tailed). When a multiple comparison was involved, one-way ANOVA was used to check the difference between the treatments groups. Wherever necessary, a difference between any of the test groups with the control was assessed using the *t*-test with Dunnett's correction. A probability of $P<0.05$ was accepted as showing a significant difference between treatments and the control values. All results are expressed as the means ± s.e.m. The potencies of the ET agonists were expressed as pEC_{50} values relative to individual maxima.

Results

The cumulative addition of ET (0.01 nM–0.3 μM) to the proximal and distal sections of the mouse colon produced a concentration-dependent contractile response, with pEC_{50} value of 9.20 ± 0.09 and E_{max} of 1.24 ± 0.08 g at 100 nM in the proximal colon and pEC_{50} value of 9.16 ± 0.09 and E_{max} of 1.36 ± 0.14 at 100 nM in distal sections, respectively ($n=8$) (Figure 1). The contractile response to ET was unaffected in the presence of TTX. The concentration–response curves to ET in the presence and absence of TTX were superimposable in both the proximal and distal segments of the colon ($n=8$, data not shown).

The addition of the ET_A receptor-selective antagonist, BMS 182874 (0.3, 3 and 30 μM) caused a rightward shift of the concentration–response curve to ET-1 in both sections of the colon, without the suppression of the maximum response. The rightward shift of the curve to ET-1 increased as the concentration of BMS 182874 increased (Figure 2a and b).

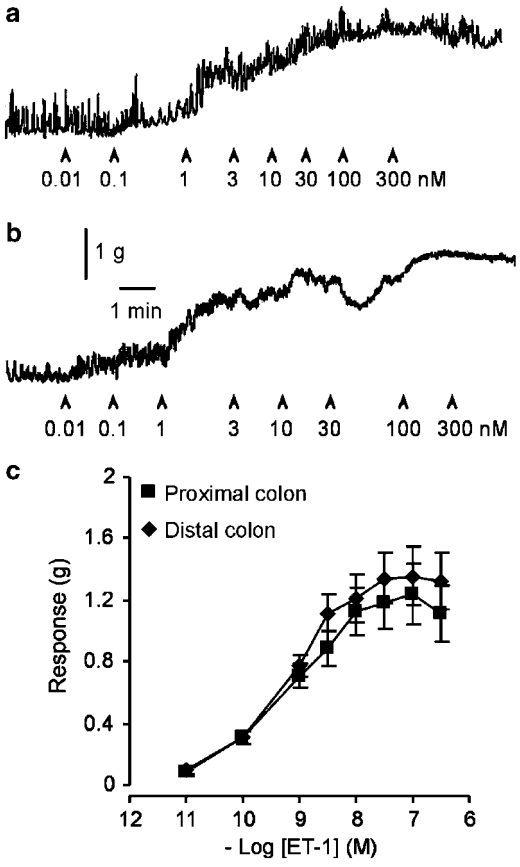

Figure 1 Representative tracing showing the contractile response induced by the cumulative addition of increasing concentrations of endothelin-1 (ET-1) (0.01 nM–0.3 μM) on the mouse (a) proximal and (b) distal colon. (c) Concentration–response curves to the cumulative addition of ET-1 (0.01 nM–0.3 μM) in the mouse proximal and distal colon ($n=8$). The graph represents the mean ± s.e.m. as taken from eight animals.

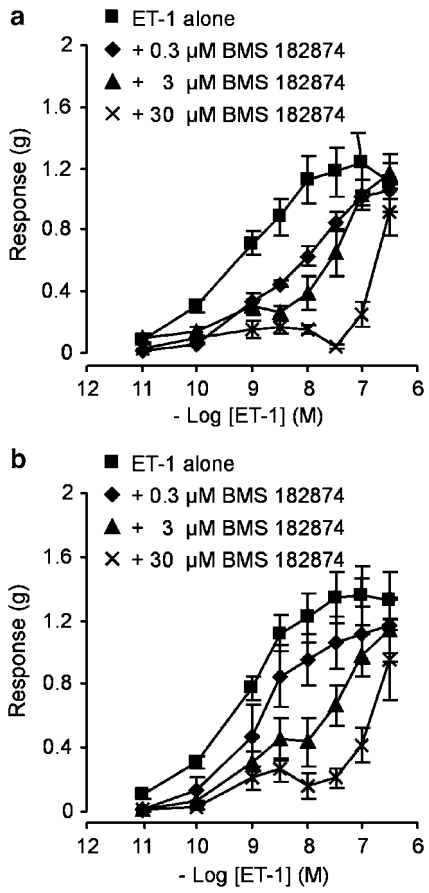

Figure 2 Antagonism of the contractile response to ET-1 in the mouse colon by BMS 182874. Concentration–response curves to endothelin-1 in the absence (ET-1 alone, $n=8$) and presence of 0.3 μM (+0.3 μM BMS 182874, $n=8$), 3 μM (+3 μM BMS 182874, $n=8$) and 30 μM (+30 μM BMS 182874, $n=8$) of BMS 182874 in the (a) proximal and (b) distal mouse colon. Each point represents the means with the s.e.m. shown by vertical bars.

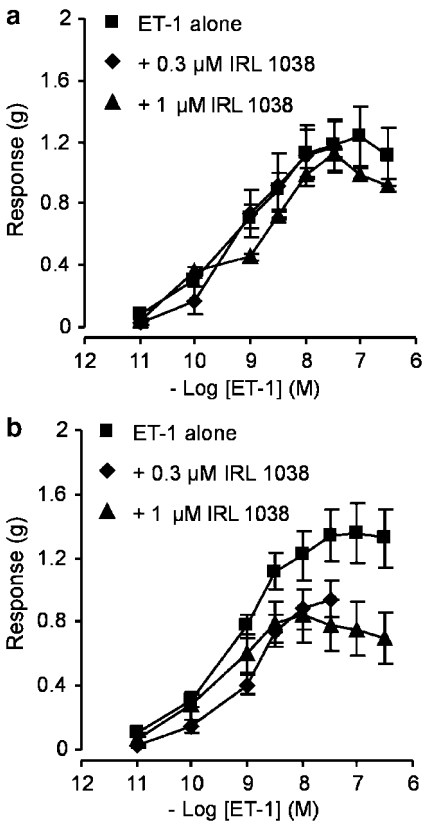

Figure 3 Antagonism of the contractile response to endothelin-1 (ET-1) in the mouse colon by IRL 1038. Concentration–response curves to endothelin-1 in the absence (ET-1 alone, $n=8$) and presence of 0.3 μM (+0.3 μM IRL 1038, $n=8$) and 1 μM (+1 μM IRL 1038, $n=8$) of IRL 1038 in the (a) proximal and (b) distal mouse colon. Each point represents the means with the s.e.m. shown by vertical bars.

The addition of the ET_B receptor antagonist, IRL 1038 (0.3 and 1 μM), did not significantly effect the response to ET-1 in the proximal sections of the colon but caused a significant decrease towards higher concentration ranges (>3 nM, $P<0.05$) in the distal colon (Figure 3a and b).

The cumulative addition of ET-1, ET-2, and ET-3 in concentrations ranging from 0.01 nM to 0.3 μM produced concentration-dependent contractions in both the proximal and distal sections of the colon (Figure 4a and b). The pEC_{50} values and E_{max} values were: 9.20±0.09 and 1.24±0.08 g at 100 nM for ET-1; 8.8±0.09 and 1.15±0.07 g at 100 nM for ET-2; 7.33±0.19 and 0.92±0.2 g at 300 nM for ET-3, ($n=4$–8) in the proximal colon and 9.16±0.09 and 1.36±0.14 g at 100 nM for ET-1; 9.08±0.07 and 1.18±0.05 g at 100 nM for ET-2; 7.9±0.25 and 1.91±0.26 g at 300 nM for ET-3 ($n=4$–8) in the distal colon. The addition of ($Ala^{1,3,11,13}$) ET and IRL1620 (0.01 nM–0.3 μM) did not produce any response in the proximal section of the colon but produced a smaller contraction in the distal sections (pEC_{50} values and E_{max} values were: 0±0 and 0±0 for ($Ala^{1,3,11,13}$) ET; 0±0 and 0±0 for IRL1620, $n=4$, in the proximal section of the colon and pEC_{50} values and E_{max} values were: 8.1±0.22 and 0.57±0.12 g at 10 nM for ($Ala^{1,3,11,13}$) ET; 7.15±0.12 and 0.62±0.10 g for IRL1620 at 10 nM, $n=4$, in distal sections of the colon).

Discussion

In the mouse, the role of ET and its receptors has previously only been demonstrated in sections taken from the mouse ileum where ET was capable of causing a dose-dependent contraction (Ishida *et al*., 1989). Moreover, it was shown that a new peptide family, ET consisting of three members in mammals, appeared to be present in mice (Saida *et al*., 1989). Of these, two ET-related genes were identified by cloning and sequence analysis of a mouse genome; one encoded a peptide identical to the porcine and human vasoconstrictor peptide ET while the other encoded a novel peptide differing from ET in three amino-acid residues. This peptide, so named 'vasoactive intestinal contractor,' was expressed in the intestine and in no other tissues or endothelial cells (Saida *et al*., 1989). To date however, ET receptor(s) have not been fully studied in murine colonic tissues.

The results from the present study showed that ET could contract both the proximal and distal sections of the mouse colon showing the existence of ET receptors in the mouse lower bowel. The receptors mediating ET contraction in both proximal and distal colon are likely to be located on the smooth muscles as the TTX did not alter response to ET. The addition of the nonpeptide ET_A receptor, selective antagonist BMS 182874 (Webb *et al*., 1995) caused a rightward shift of the concentration–response curve to ET-1 in both sections

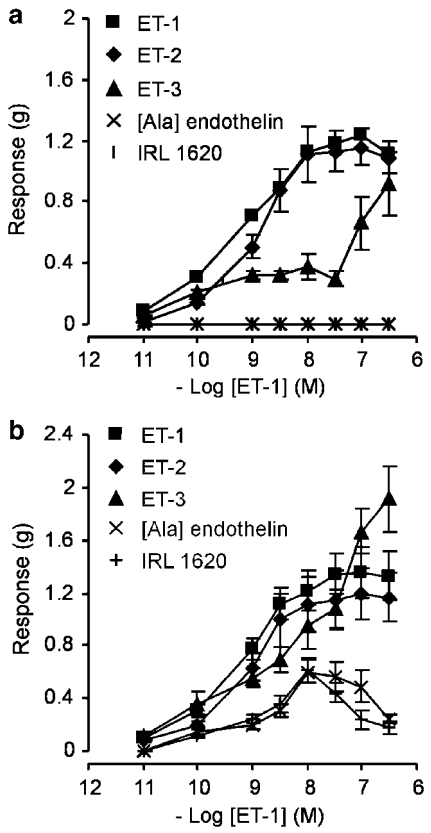

Figure 4 Graphical representation of the effect of the cumulative addition of ET-1 ($n=8$), ET-2 ($n=8$), ET-3 ($n=4$), ($Ala^{1,3,11,13}$) endothelin ($n=4$) and IRL 1620 ($n=4$) in concentrations ranging from 0.01 nM to 0.3 μM in the mouse (a) proximal and (b) distal colon. Each value represents the mean $\pm$ s.e.m. as taken from eight animals.

of the colon, without suppression of the maximum response, indicating an involvement of the ET_A receptor in the contractile response to ET-1 in both proximal and distal sections of the mouse colon.

The ET_B receptor antagonist IRL1038 (0.3–1 μM) (Masaki *et al*., 1994) did not significantly affect the response to ET-1 in the proximal colon. However, IRL 1038 caused a significant decrease in the response to ET-1 in higher concentrations (>3 nM, $P<0.05$) in the distal colon, indicting the involvement of the ET_B receptors in this section of the mouse colon. This was further supported by the ability of the two ET_B receptor agonists, ($Ala^{1,3,11,13}$) ET and IRL1620 to produce contractile responses in the distal (but not proximal sections) of the colon. Furthermore, the rank order of the potency of agonists was, ET-1 $\geqslant$ ET-2 $\gg$ ET-3 in the proximal colon and ET-1 $\geqslant$ ET-2 $\geqslant$ ET-3 in the distal colon, indicative of the presence of ET_A receptors in both the proximal and distal colon (Masaki *et al*., 1994).

Therefore, the results indicate the presence of ET receptors in the mouse colon to mediate a contraction response. It seems that ET mediates its effects predominantly *via* ET_A receptors in the proximal colon and through both ET_A and ET_B receptors in the distal section of the mouse colon. Moreover, the present study also illustrates the potential role of the various ET receptor ligands in moderating contractility in the lower bowel. With the advent of knockout mice models, the importance of the ET receptors in colonic contraction and measures of motility can now be studied (Davenport & Maguire, 2002).

The present study demonstrated that ET is highly potent in the mouse colon as it initiates the contraction response at concentrations as low as 10^{-11} M. Similar values have been obtained from studies carried out in rat stomach, jejenum, ileum and colon where concentrations of 10^{-11}–10^{-7} M ET could contract the stomach and colon of rats and also the ileum of the guinea pig (Takahashi *et al*., 1990). The potency of ET-1 observed in the present study is also comparable to the potency of ET-1 reported for contraction of the guinea-pig ileum (Ishida *et al*., 1989).

ET-1 and ET-3 peptides have been shown to be present in the gastrointestinal tract; they have been found in the rat gastric mucosa by immunoassay (Matsumoto *et al*., 1989), by *in situ* hybridization (MacCumber *et al*., 1989), and by ET binding (Takahashi *et al*., 1990). ET-3 has also been shown to play an important role in the development of the mouse enteric nervous system (Gershon, 1995). Both ET_B and ET-3 receptors are necessary to prevent the premature differentiation of crest-derived cells, which leads to agangliosis (Wu *et al*., 1999). A number of studies have shown that the pharmacological effects of exogenous ET-1 in the intestine are revealing of a potent intestinal secretagogue and an effect to increase colon contraction by direct stimulation of the smooth muscle (Brown and Smith, 1991; Moummi *et al*., 1992; Kiyohara *et al*., 1993). A study by Egidy *et al*. (2000) showed the presence of the ET_B receptors in neurons and glial cells, indicating that they may be involved in the formation of enteric neurons as has been shown in the mouse (Wu *et al*., 1999). Moreover, the various cellular localizations of the ET components suggested that they might be implicated in the modulation of intestinal motility and secretion.

There have also been reports on the distribution of ET in the human colon. Inagaki *et al*. (1994) reported ET-like immunoreactivity and binding sites for ET-1 in the human colon and competition studies have shown that there are two populations of ET receptors (Escrig *et al*., 1992). Mutations of ET_B receptors have been found in some families with Hirschsprung's disease or agangliosis (Puffenberger *et al*., 1994; Zhu *et al*., 2004).

The findings from this study therefore provide evidence for the involvement of ET and ET_A and ET_B receptors in mediating a contraction response in the mouse colon. This may suggest a role for ET in the modulation of colonic and intestinal function and pathophysiology. It would be of interest to further investigate the possibility that a pharmacological manipulation of the ET receptor subtypes might reveal a therapeutic potential.

References

BROWN, M.A. & SMITH, P.L. (1991). Endothelin – a potent stimulator of intestinal ion secretion *in vitro*. *Regulatory Peptides,* **36,** 1–19.

DAVENPORT, A.P. & MAGUIRE, J.J. (2002). Of mice and men: advances in endothelin research and first antagonist gains FDA approval. *Trends Pharmacol. Sci.,* **23,** 155–157.

EGIDY, G., JUILLERAT-JEANNERET, L., KORTH, P., BOSMAN, F.T. & PINET, F. (2000). The endothelin system in normal human colon. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **279,** G211–G222.

ESCRIG, C., BISHOP, A.E., INAGAKI, H., MOSCOSO, G., TAKAHASHI, K, VARNDELL, I.M., GHATEI, M.A., BLOOM, S.R. & POLAK, J.M. (1992). Localisation of endothelin like immunoreactivity in adult and developing human gut. *Gut,* **33,** 212–217.

GERSHON, M.D. (1995). Neural crest development. Do developing enteric neurons need endothelins? *Curr. Biol.,* **5,** 601–604.

GILLESPIE, M.N., OWASOYO, J.O., MCMURTRY, I.F. & O'BRIEN, R.F. (1986). Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. *J. Pharmacol. Exp. Ther.,* **236,** 339–343.

HICKEY, K.A., RUBANYI, G., PAUL, R.J. & HIGHSMITH, R.F. (1985). Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am. J. Physiol.,* **248,** C550–C556.

INAGAKI, N., YOSHIDA, H., MIZUTA, M., MIZUNO, N., FUJII, Y., GONOI, T., MIYAZAKI, J. & SEINO, S. (1994). Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 2679–2683.

INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K. & MASAKI, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 2863–2867.

ISHIDA, N., TSUJIOKA, K., TOMOI, M., SAIDA, K. & MITSUI, Y. (1989). Differential activities of two distinct endothelin family peptides on ileum and coronary artery. *FEBS Lett.,* **247,** 337–340.

KARNE, S., JAYAWICKREME, C.K. & LERNER, M.R. (1993). Cloning and characterization of an endothelin-3 specific receptor (ETC receptor) from *Xenopus laevis* dermal melanophores. *J. Biol. Chem.,* **268,** 19126–19133.

KASUYA, Y., KOBAYASHI, H. & UEMURA, H. (1991). Endothelin-like immunoreactivity in the nervous system of invertebrates and fish. *J. Cardiovasc. Pharmacol.,* **17,** S463–S466.

KIYOHARA, T., OKUNO, M., NAKANISHI, T., SHINOMURA, Y. & MATSUZAWA, Y. (1993). Effect of endothelin 1 on ion transport in isolated rat colon. *Gastroenterology,* **104,** 1328–1336.

MACCUMBER, M.W., ROSS, C.A., GLASER, B.M. & SNYDER, S.H. (1989). Endothelin: visualization of mRNAs by *in situ* hybridization provides evidence for local action. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 7285–7289.

MASAKI, T., VANE, J.R. & VANHOUTTE, P.M. (1994). International Union of Pharmacology nomenclature of endothelin receptors. *Pharmacol. Rev.,* **46,** 137–142.

MATSUMOTO, H., SUZUKI, N., ONDA, H. & FUJINO, M. (1989). Abundance of endothelin-3 in rat intestine, pituitary gland and brain. *Biochem. Biophys. Res. Commun.,* **164,** 74–80.

MOUMMI, C., XIE, Y., KACHUR, J.F. & AND GAGINELLA, T.S. (1992). Endothelin-1 stimulates contraction and ion transport in the rat colon: different mechanisms of action. *J. Pharmacol. Exp. Ther.,* **262,** 409–414.

O'BRIAN, R.F., ROBBINS, R.J. & MCMURTRY, I.F. (1987). Endothelial cells in culture produce vasoconstrictor substance. *J. Cell Physiol.,* **132,** 263–270.

PESKAR, B.M., NOWAK, P. & LAMBRECHT, N. (1992). Effect of prostaglandins and capsaicin on gastric vascular flow and mucosal injury in endothelin-1-treated rats. *Agents Actions Suppl.,* **37,** 85–91.

PUFFENBERGER, E.G., HOSODA, K., WASHINGTON, S.S., NAKAO, K., WIT, D., YANAGISAWA, M. & CHAKRAVART, A. (1994). A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell,* **79,** 1257–1266.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.,* **46,** 325–415.

SAIDA, K., MITSUI, Y. & ISHIDA, N. (1989). A novel peptide, vasoactive intestinal contractor, of a new (endothelin) peptide family. Molecular cloning, expression, and biological activity. *J. Biol. Chem.,* **264,** 14613–14616.

SAKURAI, T., YANAGISAWA, M. & MASAKI, T. (1992). Molecular characterization of endothelin receptors. *Trends Pharmacol. Sci.,* **13,** 103–108.

TAKAHASHI, K., GHATEI, M.A., LAM, H.C. & O'HALLORAM, D.J. (1990). Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia,* **33,** 1200–1202.

TEKIN, E., TANERI, F., ERSOY, E., BOZKURT, S., YAVUZER, R., ERCAN, S. & OGUZ, M. (1999). Ileal and colonic contractions by endothelin-1 in experimentally induced paralytic ileus in rats. *Gen. Pharmacol.,* **32,** 631–635.

WALLACE, J.L., KEENAN, C.M., MACNAUGHTON, W.K. & MCKNIGHT, G.W. (1989). Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach. *Eur. J. Pharmacol.,* **167,** 41–47.

WEBB, M.L., BIRD, J.E., LIU, E.C., ROSE, P.M., SERAFINO, R., STEIN, P.D. & MORRELAND, S. (1995). BMS-182874 is a selective, nonpeptide endothelin ET_A receptor antagonist. *J. Pharmacol. Exp. Ther.,* **272,** 1124–1134.

WU, J.J., CHEN, J.X., ROTHMAN, T.P. & GERSHON, M.D. (1999). Inhibition of *in vitro* enteric neuronal development by endothelin-3: mediation by endothelin B receptors. *Development,* **126,** 1161–1173.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature,* **332,** 411–415.

ZHU, L., LEE, H.O., JORDAN, C.S., CANTRELL, V.A., SOUTHARD-SMITH, E.M. & SHIN, M.K. (2004). Spatiotemporal regulation of endothelin receptor-B by SOX10 in neural crest-derived enteric neuron precursors. *Nat. Genet.,* **36,** 732–737.

(Received August 12, 2005
Revised October 20, 2005
Accepted December 13, 2005
Published online 23 January 2006)