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Developmental changes in response to endothelins and receptor subtypes of isolated rat duodenum

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Abstract

The response of isolated duodenum to endothelin-1, -3 and IRL 1620 (Suc-[Glu^{9},Ala^{11,15}]endothelin-1 (8–21)), a selective endothelin ET_B receptor agonist, was studied in both neonatal (1-week-old) and adult rats by recording mechanical activity isotonically. Endothelin-1, -3 and IRL 1620 (1–100 nM) elicited sustained contraction of neonatal duodenum, in a concentration-dependent manner, with a potency order of endothelin-1 = endothelin-3 > IRL 1620. The response to endothelin-1 and -3 (10–1000 nM) of adult duodenum was biphasic, i.e., transient relaxation followed by contraction, with a potency order of endothelin-1 > endothelin-3. The contractile response to endothelin-1 of adult but not neonatal duodenum was significantly antagonized by pretreatment with FR139317 (1 μM), an endothelin ET_A receptor antagonist. An endothelin ET_B receptor antagonist, RES-701-1 (3 μM), weakly antagonized the IRL 1620-induced contraction of neonatal duodenum. However, RES-701-1 (10 μM) did not affect the response to endothelin-1 of either adult or neonatal duodenum. These results indicate that the duodenal response to endothelins changes from a sustained contraction in neonates to a biphasic response in adults. The contractile response to endothelins of neonatal duodenum is suggested to be mediated through endothelin ET_B receptors or possibly RES-701-1-resistant ET_B receptor subtypes and contraction of adult duodenum through endothelin ET_A receptors. The mechanism of the endothelin-induced response of duodenum was also studied.

Keywords: Endothelin; Endothelin receptor subtype; IRL 1620; FR139317; RES-701-1; Duodenum; (Rat)

1. Introduction

Endothelin was originally isolated from porcine endothelial cells as a peptide to induce hypertension (Yanagisawa et al., 1988). Endothelins and endothelin receptors are distributed in various organs, including the gastrointestinal tract (Matsumoto et al., 1989; Takahashi et al., 1990; Arai et al., 1990; Sakurai et al., 1990, 1991). Endothelin-1 induces a biphasic response, i.e., transient relaxation followed by sustained contraction, in guinea-pig ileum (Lin and Lee, 1990, 1992; Miasiro and Paiva, 1990, 1992). The response of the longitudinal muscle of the rat gastric fundus is reported to be contraction alone (Fulginiti et al., 1993; Shimomura et al., 1994).

We have studied developmental changes in the response of rat duodenum to various neuropeptides and biologically active substances such as thyrotropin-releasing hormone (TRH), neuropeptide Y, ATP and nicotine (Tonoue et al., 1981; Furukawa and Nomoto, 1989; Irie et al., 1993, 1994). The duodenal response to TRH, ATP and nicotine was contraction in the neonatal period, but relaxation in the adult (Tonoue et al., 1979, 1981; Furukawa and Nomoto, 1989; Irie et al., 1994). The response of duodenum to neuropeptide Y changes from biphasic contraction to monophasic contraction during development (Irie et al., 1993). In this paper, we investigated the response to endothelin-1 and -3 and IRL 1620, a selective endothelin ET_B receptor agonist (Takai et al., 1992), by isolated duodenum from neonatal and adult rats, and studied the receptor subtypes mediating the duodenal response to endothelin-1. We found that the response of rat duodenum to endothelin-1 changes from an ET_B

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receptor-mediated contraction in neonates to an ET_A receptor-mediated contraction preceded by transient relaxation in adults.

2. Materials and methods

2.1. Preparation and measurement of mechanical activity of duodenum

Male Wistar-Imamichi rats aged 1 and 9–15 weeks were used. Neonatal rats were killed by decapitation and adult rats stunned by a blow to the head and bled. The duodenum of each animal was dissected out, and the proximal portion, but not the bulb (1.5 cm for neonatal rats and 2.2 cm for adult rats), was suspended in an organ bath containing 5 ml of modified Locke solution (composition in mM: NaCl 154, KCl 4.02, $CaCl_2$ 1.36, $MgCl_2$ 0.9, $NaHCO_3$ 2.97, glucose 5.56, pH 7.0) saturated with 95% O_2 and 5% CO_2 and maintained at 37°C with continuous bubbling of air. The mechanical activity of the segments under a load of 0.5 g (neonate) and 1 g (adult) was recorded with an isotonic transducer (Nihonkohden TD-112S) on a polygraphic recorder (Nihonkohden WT-645G). After a 1-h equilibration, the duodenum was exposed to 50 mM KCl (hypertonic supply) for 2 min repeatedly (2–3 times) and then the response to endothelins was examined. The data are expressed as percentage of the 50 mM KCl-induced contraction (tonic phase). Student's t-test was used to assess the significance of the difference between group means, and $P < 0.05$ was considered significant.

2.2. Drugs

The following drugs were used: endothelin-1, endothelin-3 and IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1 (8–21)) (Peptide Institute, Osaka, Japan), N^G-nitro-L-arginine methyl ester and apamin (Sigma), FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl] amino-3-[3-(1-methyl-1*H*-iodoyl)]propionyl]amino-3-(2-pyridyl)-propionic acid)) (kindly provided by Fujisawa Pharmaceutical Co., Osaka, Japan), RES-701-1 (cyclic [Gly^1-Asp^9][Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp]) (kindly provided by Kyowa Hakko Kogyo, Tokyo, Japan) and tetrodotoxin (Sankyo, Tokyo, Japan). Endothelins and IRL 1620 were dissolved in 0.7% acetic acid to make 100 μM and diluted to adequate concentration with physiological saline. RES-701-1 was dissolved in dimethyl sulfoxide and a 5- to 50-μl aliquot was added to the bath. Other drugs were dissolved in physiological saline, which was added to the bath as a 30- or 100-μl aliquot.

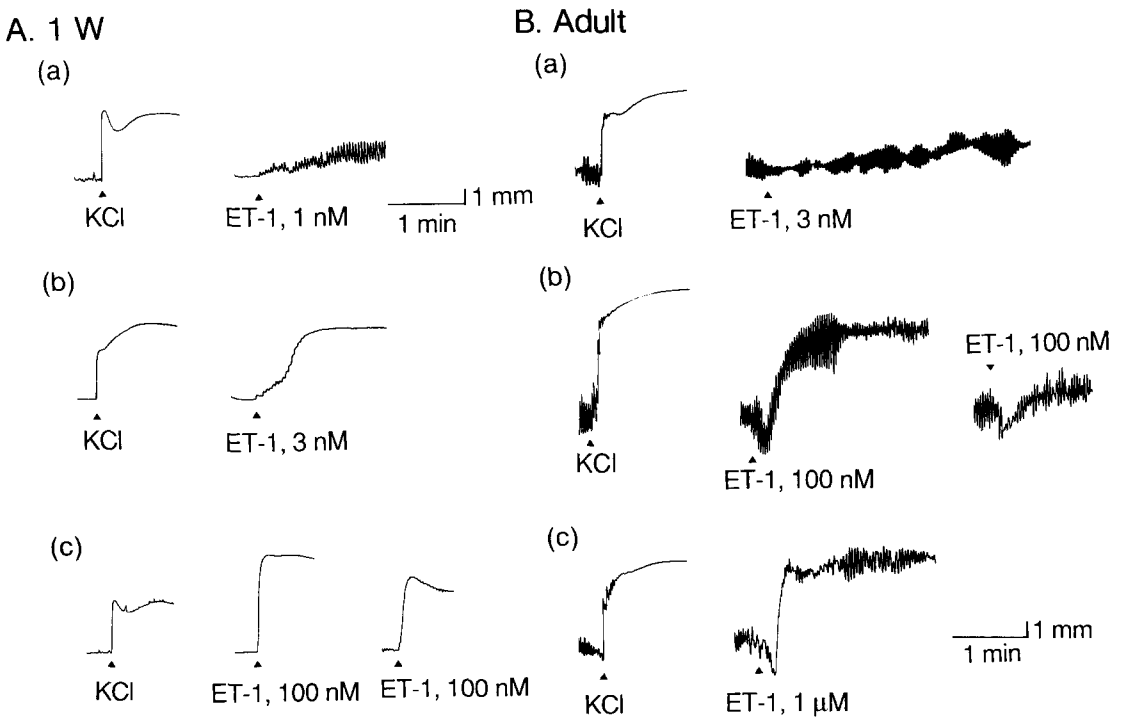

Fig. 1. Response to endothelin (ET)-1 of isolated rat duodenum in neonatal (A) and adult rats (B). A 45-min recovery period was taken before the second exposure to endothelin-1 in A(c) and B(b). Concentration of KCl was 50 mM.

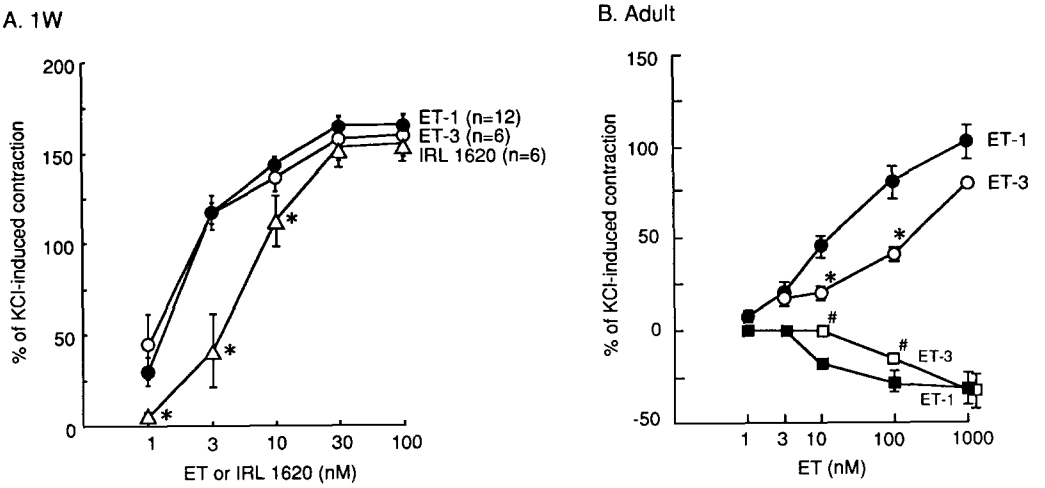

Fig. 2. Concentration-response curves for endothelin (ET)-1 and -3 and IRL 1620 in neonatal (A) and adult duodenum (B). Concentration-response curves were constructed by cumulative method in A and by single concentration-response in B. Concentration of KCl was 50 mM. Data shown are means ± S.E.M. A negative value means relaxation. Number of preparations in B was 4–8 for each concentration. * $P < 0.05$ vs endothelin-1-induced contraction and # $P < 0.05$ vs. endothelin-1-induced relaxation for each concentration by Student's t-test.

3. Results

3.1. Response of neonatal duodenum to endothelins and IRL 1620

Endothelin-1 elicited contraction of neonatal duodenum in a concentration-dependent manner (Figs. 1A and 2A). A lower concentration of endothelin-1 induced an increase in spontaneous activity and a slowly developing contraction, which reached a plateau level in 3–5 min (Fig. 1A(a)). A higher concentration of endothelin-1 elicited a steep contraction (Fig. 1A(c)). Since repeated exposure to endothelins led to tachyphylaxis (Fig. 1A(c), second response to endothelin-1 was 64 ± 4% ($n = 6$) of first response), each preparation was used for only one series of cumulative concentration-response curves, which were compared to the 50 mM KCl-induced contraction. The response of neonatal duodenum to endothelin-3 was not significantly different from that to endothelin-1 (Fig. 2A). The response to IRL 1620 was smaller than that to endothelin-1 in lower concentrations (1–10 μM), but of similar magnitude at 30–100 nM (Fig. 2A). The pD_2 values, calculated from concentration-response curves, for endothelin-1, -3 and IRL 1620 were 8.70, 8.77 and 8.42 respectively.

3.2. Response of adult duodenum to endothelins

Endothelin-1 elicited a biphasic response in adult duodenum, i.e., transient relaxation followed by sustained contraction, although the early relaxation phase was not observed with a lower concentration of endothelin-1 (Fig. 1B). The contractile phase induced by endothelin-1 was composed of two components, i.e., phasic and tonic contractions (Figs. 1B(c) and 4B). A strong tachyphylaxis was produced by a repeated exposure to endothelins (Fig. 1B(b)). The contraction due

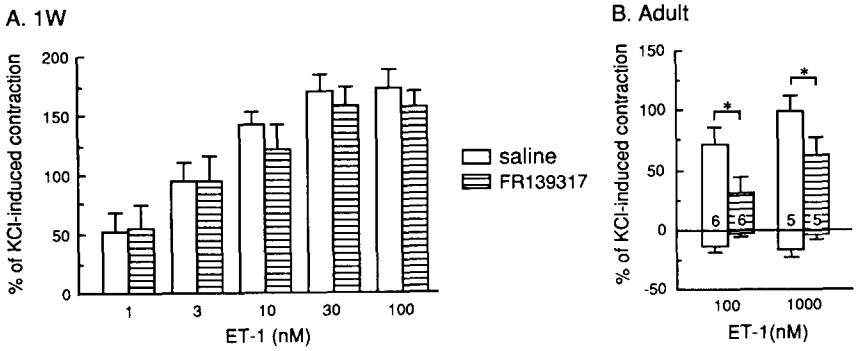

Fig. 3. Effect of FR139317, an endothelin ET_A receptor antagonist, on the endothelin (ET)-1-induced response of neonatal (A) and adult duodenum (B). FR139317 or vehicle (saline) was added to the bath 15 min prior to endothelin-1. Number of preparations in A was 7 for saline group and 8 for FR139317 group. * $P < 0.05$ by Student's t-test.

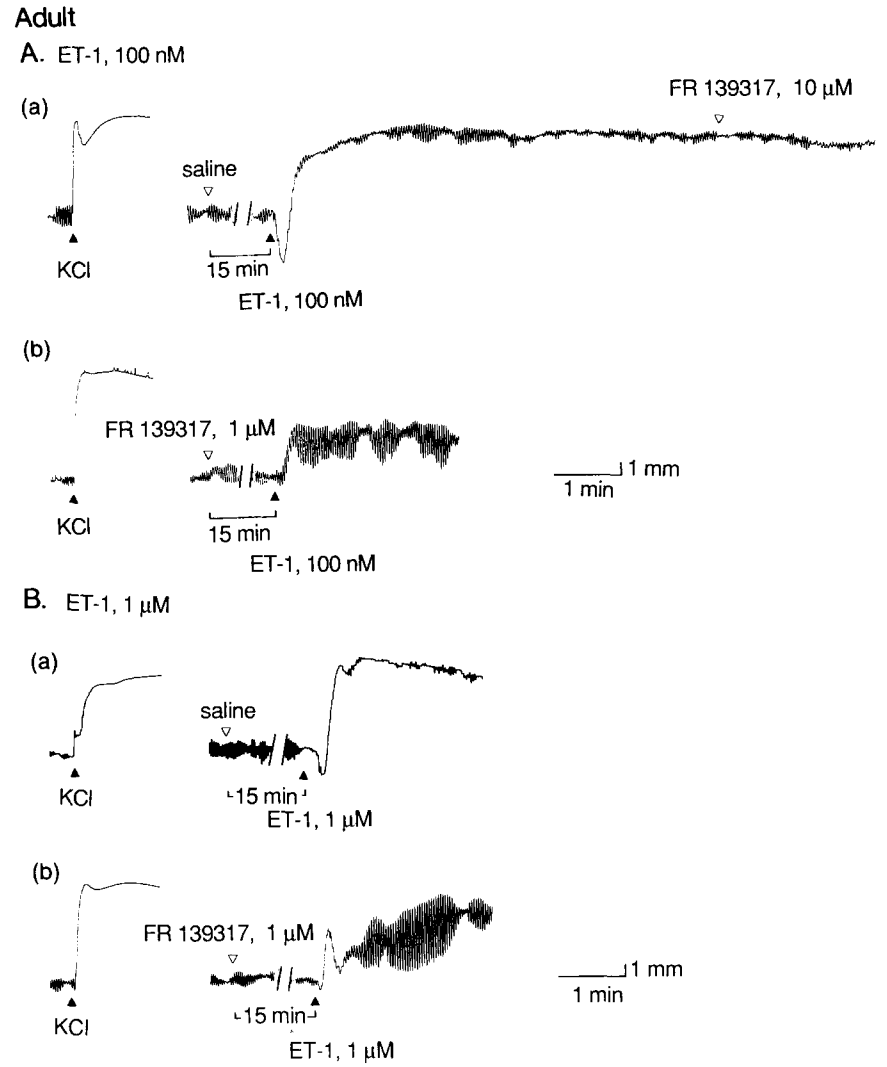

Fig. 4. Effect of FR139317 on the duodenal response to endothelin (ET)-1 (100 nM in A and 1 μM in B) in adult rat. FR139317 was added to the bath 15 min prior to endothelin-1 or at the plateau of the contraction. Concentration of KCl was 50 mM.

to the second exposure to endothelin-1 was 26 ± 5% ($n = 4$) of that of the first exposure, whereas 67 ± 13% ($n = 4$) remained at a relaxant response (Fig. 1B(b)). We tested only one concentration of endothelin in each preparation for further studies, and used the 50 mM KCl-induced contraction as a reference. The re-

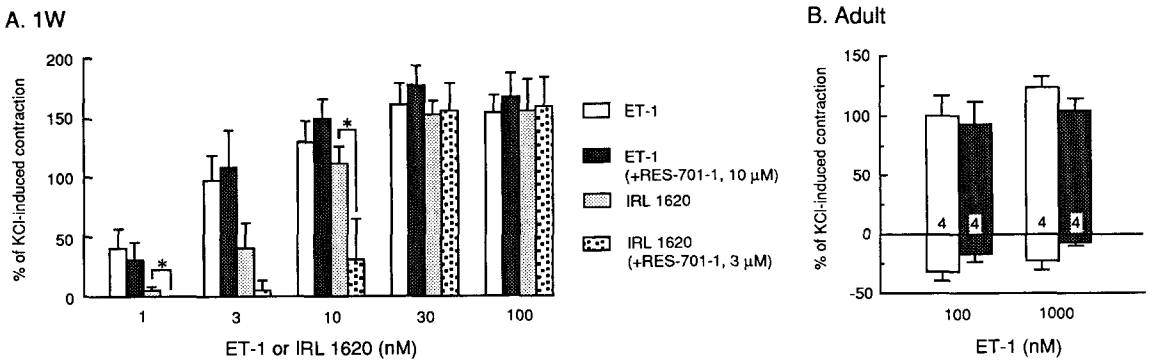

Fig. 5. Effect of RES-701-1 on the endothelin (ET)-1- and IRL 1620-induced responses of neonatal duodenum (A) and adult duodenum (B). RES-701-1 was added to the bath 15 min prior to the agonists. Number of preparations in A was 5 or 6 for each group. * $P < 0.05$ by Student's t-test.

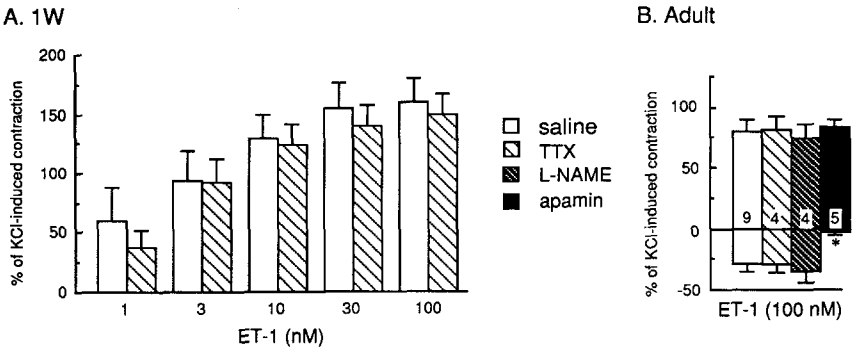

Fig. 6. Effect of tetrodotoxin (1 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and apamin (0.5 μM) on endothelin (ET)-1-induced responses of neonatal duodenum (A) and adult duodenum (B). Tetrodotoxin, N^G-nitro-L-arginine methyl ester and apamin were added to the bath 15 min prior to endothelin-1. Number of preparations in A was 4 for each group. * $P < 0.05$ vs. endothelin-1-induced relaxation in saline control by Student's t-test.

sponse of adult duodenum to endothelin-3 was also biphasic, but significantly smaller than that elicited by endothelin-1 (Fig. 2B). IRL 1620 (100 nM) induced a small transient relaxation without contraction of adult duodenum (data not shown). The negative logarithms of the concentrations which produced 50% of the KCl-induced contraction were 7.87 and 6.78 for endothelin-1 and -3 respectively.

3.3. Effects of endothelin receptor antagonists on endothelin-1- and IRL 1620-induced responses of duodenum

The effects of endothelin ET_A and ET_B receptor antagonists were examined on the endothelin-1-induced response of duodenum from both neonatal and adult rats. The response of neonatal duodenum to endothelin-1 was not affected by pretreatment with FR139317 (1 μM, 15 min), an endothelin ET_A antagonist (Sogabe et al., 1993) (Fig. 3A). In adult rat duodenum, pretreatment with FR139317 (1 μM, 15 min) significantly antagonized the contractile response of duodenum to endothelin-1 (Figs. 3B and 4). FR139317, however, was ineffective when it was added to the bath during the contraction of duodenum by endothelin-1 (Fig. 4A(a)). The phasic contraction in response to a higher concentration (1 μM) of endothelin-1 was rather resistant to FR139317 (Fig. 4B(b)). The relaxation induced by endothelin-1 was smaller (but not statistically significant) in the FR139317- and RES-701-1-pretreated preparation than in the control duodenum (Figs. 3B and 5B).

The duodenal response to endothelin-1 was not significantly antagonized by RES-701-1 (10 μM, 15 min), an endothelin ET_B antagonist (Tanaka et al., 1994), in either neonatal or adult duodenum (Fig. 5). RES-701-1 (3 μM), however, significantly antagonized the contractile response to lower (1 and 10 μM) but not higher concentrations of IRL 1620 in neonatal duodenum (Fig. 5A). This concentration (3 μM) of RES-701-1 was sufficient to abolish the endothelin ET_B receptor-mediated relaxant response to 0.1 μM endothelin-3 of rat thoracic aorta precontracted by 0.1 μM norepinephrine (data not shown).

3.4. Mechanism of duodenal response to endothelin-1

The mechanism of duodenal response to endothelin-1 was examined by using several drugs. Tetrodotoxin (1 μM, 15 min) did not affect the response of either neonatal or adult duodenum to endothelin-1 (Fig. 6), whereas the concentration of tetrodotoxin used was sufficient to inhibit the duodenal response induced by nicotine and some other drugs in both neonatal and adult rats (Tonoue et al., 1979; Irie et al., 1994).

Since endogenous nitric oxide (NO) was reported to be involved in endothelin-induced vasodilatation, an NO synthase inhibitor was examined to determine a possible role of NO in the relaxant response of adult rat duodenum to endothelin-1. Neither the relaxant nor the contractile response of duodenum to endothelin-1 was inhibited by pretreatment with N^G-nitro-L-arginine methyl ester (100 μM, 15 min, Fig. 6B). This concentration of N^G-nitro-L-arginine significantly inhibited the relaxant response of adult duodenum to nicotine (Irie et al., 1991).

Apamin (0.5 μM, 15 min), a Ca^{2+}-activated K^+ channel blocker (Hugues et al., 1982), significantly blocked the endothelin-1-induced relaxation but not the contraction of adult duodenum (Fig. 6B). A cyclooxygenase inhibitor, indomethacin (10 μM, 30 min), did not inhibit the contractile response of adult duodenum to endothelin-1 (data not shown).

4. Discussion

Immunoreactive endothelin-1 and -3 were found in the entire region of the gastrointestinal tract in rats (Matsumoto et al., 1989; Takahashi et al., 1990). Mes-

senger RNA for endothelin ET_A and ET_B receptors was reported to be expressed in the rat and bovine gastrointestinal tract (Arai et al., 1990; Sakurai et al., 1990). Therefore, endogenous endothelins may play an important physiological role in gastrointestinal activities. Endothelin receptors are classified into ET_A and ET_B. The former is selectively activated by endothelin-1 rather than by endothelin-3, and the latter is equally activated by endothelin-1 and endothelin-3 (Arai et al., 1990; Sakurai et al., 1990). Recently, a subclassification of endothelin ET_A and ET_B receptors has been proposed, based on their differential sensitivity to endothelin receptor antagonists (Sudjarwo et al., 1993,1994; Hori et al., 1994). The endothelin ET_B receptors may be subclassified into RES-701-1-sensitive and -resistant ET_B receptor subtypes.

The response of adult rat duodenum to endothelin-1 was biphasic, i.e., transient relaxation followed by contraction, consistent with previous reports on guinea-pig ileum (Lin and Lee, 1990; Miasiro and Paiva, 1990). Neonatal duodenum, however, lacked the relaxant phase. Thus we add a new example of developmental changes of duodenal responses to biologically active substances. To explain why these substances contract duodenum in neonatal rats but relax it in adult rats, we speculated that the predominant innervation in rat duodenum changes developmentally from excitatory cholinergic nerves to inhibitory non-adrenergic, non-cholinergic nerves (Irie et al., 1994). The contraction of duodenum in response to endothelins was greater than that elicited by high K^+ depolarization in neonates. The increased sensitivity of neonatal duodenum to endothelins is possibly due to endothelin-induced increases in the Ca^{2+} sensitivity of contractile elements, as was suggested for other receptor agonists which modulate Ca^{2+} sensitivity in smooth muscle (Karaki, 1989; Somlyo and Himpens, 1989; Hori et al., 1993).

Our finding that the potency of endothelin-3 for inducing contraction of neonatal duodenum was equal to that of endothelin-1 suggests that endothelin-induced contraction of neonatal duodenum is mediated through endothelin ET_B receptors. This is supported by the fact that a selective endothelin ET_B receptor agonist, IRL 1620, also induced contraction of neonatal duodenum. However, RES-701-1 only weakly antagonized the IRL 1620-induced contraction and failed to antagonize the endothelin-1-induced contraction. This may not to be due to the insufficient concentration of RES-701-1, because this concentration of RES-701-1 antagonized the endothelin ET_B receptor-mediated relaxation of rat thoracic aorta elicited by endothelin-3 in our study (not shown). Also, 5 μM RES-701-1 is reported to antagonize the endothelin ET_B receptor-mediated increase in intracellular Ca^{2+} concentration in COS-7 cells (Tanaka et al., 1994). Therefore, the endothelin-induced contraction of neonatal duodenum may act through RES-701-1-resistant endothelin ET_B receptor subtypes.

The relative potency of endothelins for eliciting contraction of adult rat duodenum was endothelin-1 > endothelin-3, and the tonic phase of the endothelin-1-induced contraction of adult duodenum was significantly antagonized by pretreatment with FR139317. These results indicate that the tonic phase of endothelin-induced contraction of adult duodenum is mediated through endothelin ET_A receptors. As FR139317 was ineffective when it was added to the bath during the contraction of duodenum by endothelin-1, the binding of endothelin-1 to the endothelin ET_A receptor seems to be very strong, which explains the strong tachyphylaxis produced by endothelins, especially those involved in the contractile response of adult duodenum. Since the phasic contraction elicited by 1 μM endothelin-1 was rather resistant to FR139317, phasic and tonic contractile responses may be mediated by different subclasses of endothelin ET_A receptors.

The potency order (endothelin-1 > endothelin-3 $\gg$ IRL 1620) for endothelin-induced relaxation suggests the mediation of endothelin ET_A receptors. However, the relaxation was antagonized by both FR139317 and RES-701-1. Further studies are required to resolve this discrepancy in the relaxant response of rat adult duodenum.

These results indicate that the endothelin receptor subtype mediating contractile response changes from neonatal ET_B to adult ET_A. However, the mechanism of developmental change of endothelin receptor subtypes in duodenum is not known. It should be pointed out that Eguchi et al. (1994) have shown that the endothelin receptor subtype of vascular smooth muscle cells in the growth stage (ET_B) is different from that of the contractile (i.e., differentiated) stage (ET_A). The change in endothelin receptor subtype could be confirmed by receptor binding studies and by investigating messenger RNA for endothelin receptors in duodenum.

The duodenal response to endothelin-1 was not affected by pretreatment with tetrodotoxin in either neonatal or adult rats, consistent with the intestinal response to endothelin-1 in guinea-pig ileum (Lin and Lee, 1990; Miasiro and Paiva, 1990), indicating that endothelin-1 acts on smooth muscle cells. Blockade of endothelin-1-induced relaxation by apamin suggests the involvement of the Ca^{2+}-activated K^+ channel in the relaxant response to endothelins, as observed in guinea-pig ileum (Lin and Lee, 1992). Since an inhibitor for NO synthase had no effect on the endothelin-1-induced relaxation of duodenum, the intestinal relaxation induced by endothelins seems to be independent of the NO pathway. Although prostaglandin is reported to be involved in the endothelin-induced contraction of rat gastric fundus (Shimomura et al., 1994),

prostaglandin plays no role in the duodenal contraction elicited by endothelin-1, consistent with the case of guinea-pig ileum (Lin and Lee, 1990).

In conclusion, the present study shows that endothelin induces sustained contraction in neonatal duodenum, whereas it elicits a transient relaxation followed by contraction in adult rat duodenum, and that endothelin contracts rat duodenum through endothelin ET_B receptors in the neonate but through endothelin ET_A receptors in the adult.

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