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Differential activities of two distinct endothelin family peptides on ileum and coronary artery

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A synthesized mouse vasoactive intestinal contractor peptide, which belongs to a novel member of the endothelin family, induced a prolonged contraction in mouse ileum as well as porcine coronary artery in vitro. Studies comparing the effects of vasoactive intestinal contractor and endothelin on different tissues revealed that the maximum ileum contraction of vasoactive intestinal contractor was much higher than that of endothelin in both guinea pig and mouse systems, but that the vasoconstriction activity of vasoactive intestinal contractor was weaker than that of endothelin in porcine artery. These resuts show that vasoactive intestinal contractor might be a novel gastrointestinal hormone.

Vasoactive intestinal contractor; Endothelin family; Intestine contraction; Ca^{2+}, extracellular; Gastrointestinal hormone

1. INTRODUCTION

A growing number of regulatory peptides have been newly identified by using gastrointestinal contraction as a non-specific assay for detecting unknown neuropeptides from various tissues. However, only a few of the cases reported have been genuine gastrointestinal hormones in respect of their expression sites and activities. Vasoactive intestinal polypeptide (VIP), isolated from porcine intestine, has biological actions including vasodilation and relaxant activity on the gastrointestinal system [1,2]. Cholecystokinin, a substance that caused contraction of the gall bladder, was isolated from duodenal mucosa [3,4]. Motilin, a 22-amino acid polypeptide isolated from porcine gut, stimulates fundus contraction [5].

Recently, we found a structurally related endothelin family (unpublished). The endothelin family consists, at present, of three peptides: endothelin [6], vasoactive intestinal contractor (VIC), and endothelin homologous peptide [7]. Endothelin (ET) is an endothelial cell derived vasoconstrictor peptide [6,8], which consists of 21 amino acid residues with 2 sets of intrachain disulfide-bonds. The novel peptide, VIC, which differs from ET in 3 amino acid residues (nos. 4, 6, 7 in 21 residues), has been cloned and sequenced from the mouse genome by us. Then the position of the cysteine residues was perfectly conserved in the VIC; in vivo pressor activities of synthetic VIC were confirmed as those of ET. Furthermore, Northern blot hybridization indicated that the VIC probe hybridized with a transcript in mouse intestine, but not in other mouse tissues or endothelial cells (Saida, K., Mitsui, Y. and Ishida, N., unpublished).

To investigate the activity of this novel peptide, VIC, we have studied the contractile effect of synthetic VIC on isolated mouse and guinea pig ileum, and porcine coronary artery, and compared its effect with that of endothelin. Here we show that VIC has a stronger effect on intestine contraction than ET and that VIC is a much less potent

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Abbreviations: VIC, vasoactive intestinal constrictor; ET, endothelin; Ach, acetylcholine

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vasoconstrictor than ET. In the presence of a Ca^{2+} channel blocker, VIC- and ET-induced contractions of guinea pig ileum are inhibited. These results are discussed in conjugation with the role of VIC as a gastrointestinal hormone and its biological actions.

2. MATERIALS AND METHODS

2.1. *Synthesis of VIC*

According to the primary structure predicted from the nucleotide sequence, VIC was made using a solid-phase peptide synthesizer. The purity of the final product was checked by analytical HPLC and amino acid analysis. The disulfide-bond topology of synthetic VIC was the same as ET, judging from HPLC profiles.

2.2. *Assay of in vitro vasoconstriction*

Right and left coronary arteries were isolated from adult porcine hearts, which were brought in ice from a slaughter house within 2 h of being killed. Parts of coronary arteries were cut into 2 × 10 mm helical strips with the intima denuded by being rubbed with a small swab, and suspended in 5 ml glass chambers filled with Krebs-Henseleit solution (118 [mM] NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgSO_4$, 10 glucose) at 37°C and gassed with 95% O_2/5% CO_2. Arterial strips were equilibrated at a passive tension of 2 *g* for 12 h. The constriction was measured as the increase in isometric tension with force-displacement transducers (model VL-10 GR, Minebea Co., Ltd).

2.3. *Assay of in vitro ileum contraction*

The animals used were mice (ICR, male) and guinea pigs (male). The ileum samples were prepared by cutting out the tract about 10 cm upstream from the cecum. Ilea, obtained from nonfasted and decapitated animals were immediately suspended in a siliconized organ bath (25 ml) that contained a modified Tyrode solution (136.8 [mM] NaCl, 2.7 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $NaHPO_4$, 11.9 $NaHCO_3$ and 5.5 glucose) at 37°C (mouse) and 27°C (guinea pig). One end was attached to a rigid support and the free end to a lever connected via a spring to a force-displacement transducer for measuring contractions. The load on the ileum was set at 0.5 *g*. The solution was bubbled with 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate for about 1 h and regular contractions were elicited by 10^{-6} M acetylcholine (mouse) and 10^{-7} M acetylcholine (guinea pig). The magnitude of a contraction was expressed as a percentage of the 10^{-5} M acetylcholine (mouse) and 10^{-6} M acetylcholine (guinea pig)-induced contraction (100%).

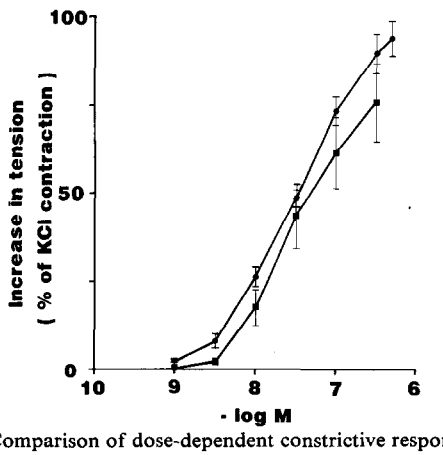

Fig.1. Comparison of dose-dependent constrictive responses of isolated aortic strips to synthetic ET (n = 7) (●) and VIC (n = 5) (■). Each point shows the mean ± SE. Vertical axis shows percentages compared to the maximum tension of 60 mM KCl-induced constrictions.

3. RESULTS

3.1. *A comparison of vasoconstriction caused by VIC and ET*

Despite the slow onset, the long lasting vasoconstriction activity of synthetic VIC was consistent

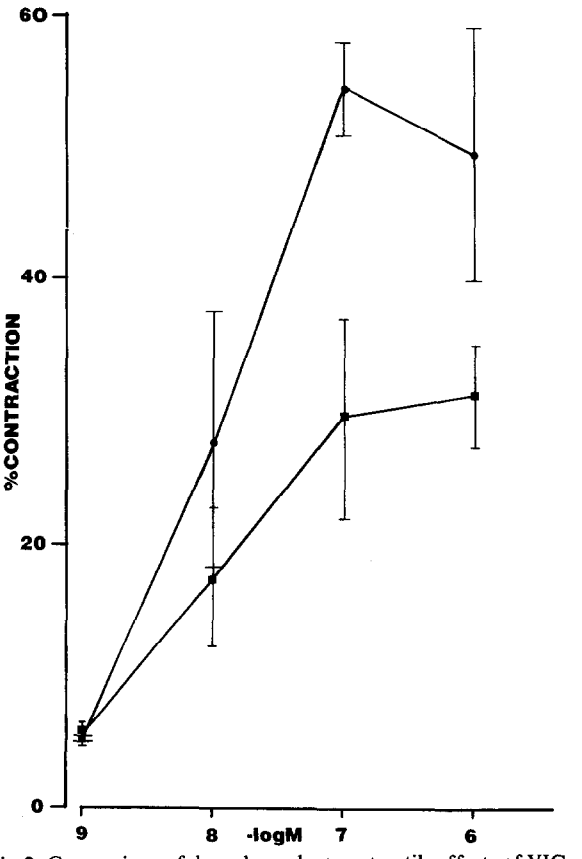

Fig.2. Comparison of dose-dependent contractile effects of VIC (●) and endothelin (■) on mouse ileum. Each point represents the mean ± SE (n = 3). The magnitude of a contraction was expressed as a percentage of the 10^{-5} M acetylcholine-induced contraction.

with that of synthetic ET on porcine coronary artery strips in vitro, the maximum tension caused by ET was much higher than that caused by VIC. In addition, the estimated EC_{50} value of ET (3.5×10^{-8} M) was lower than that of VIC (4.8×10^{-8} M). These results indicate that VIC is a much less potent vasoconstrictor than ET in porcine artery.

3.2. *A comparison of ileum contraction caused by VIC and ET*

A comparison of dose-response contraction in mouse ileum caused by VIC and ET is shown in fig.2. The estimated EC_{50} value of VIC and ET was almost the same: 8.4×10^{-9} M and 7.2×10^{-9} M, respectively. But, the maximum VIC contraction was 1.8 times higher than the maximum ET contraction, i.e. $49.3 \pm 9.5\%$ (10^{-6} M VIC) and $31.1 \pm 3.9\%$ ($\pm$ SE) (10^{-6} M ET), respectively.

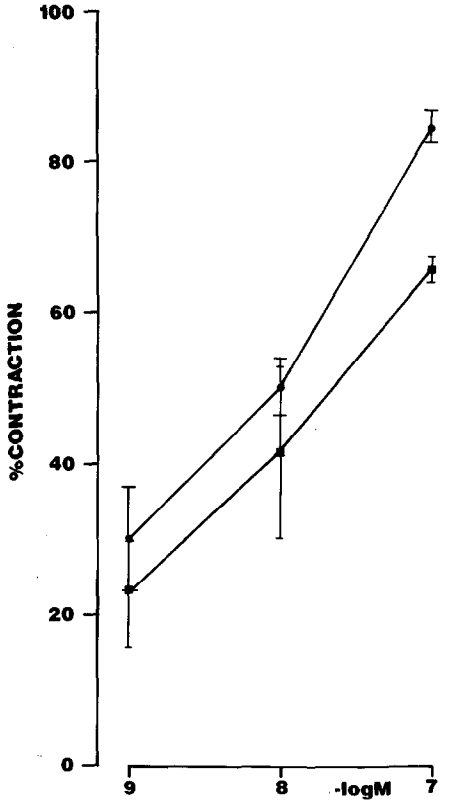

Fig.3. Comparison of dose-dependent contractile effects of VIC (●) and endothelin (■) on guinea pig ileum. Each point shows the mean ± SE ($n = 3$). The contraction was expressed as a percentage of the 10^{-6} M acetylcholine-induced contraction.

The same contractile profile of VIC and ET was also observed in guinea pig ileum, as shown in fig.3. The estimated EC_{50} values in the guinea pig assay of VIC and ET were 3.5×10^{-9} M and 4.0×10^{-9} M, respectively, but the maximum contractions of these two peptides were very distinctive, i.e. $84.6 \pm 2.1\%$ (10^{-7} M VIC) and $65.9 \pm 1.4\%$ (10^{-7} M ET). These data show that VIC is a much more potent contractor than ET in both guinea pig and mouse ileum. The slow-onset, long lasting ileum contractor activity of synthetic VIC is very similar to the vasoconstriction activity of synthetic ET and VIC in porcine coronary artery strips in vitro. About 40 min was required after contraction for ileum to return to the base-line levels. Little transient relaxation was caused by VIC input, but the biological significance of this phenomenon is unclear. VIC-induced contraction was also characteristically difficult to wash out. The maximal contraction of both 10^{-8} M VIC and 10^{-8} M ET at the same time was almost the same level as the mean magnitude of each substance-induced contraction separately (not shown).

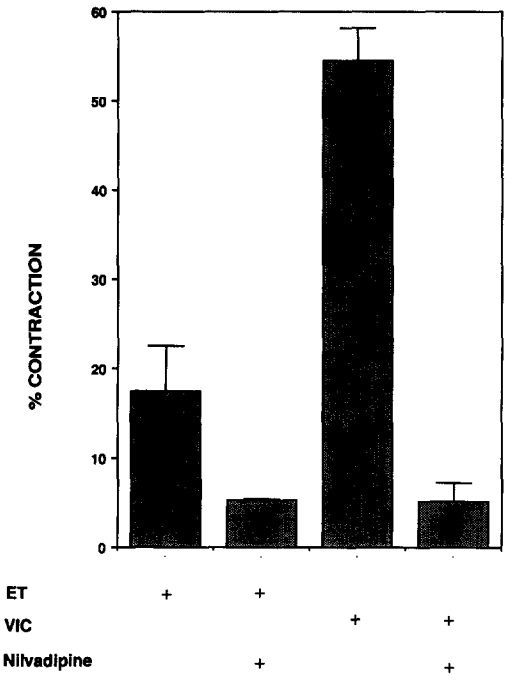

Fig.4. Inhibition of guinea pig ileum contraction by Ca^{2+} channel blocker (10^{-6} M nilvadipine [9]) to 10^{-8} M ET and 10^{-7} M VIC. Each bar indicates the % contraction which represents the mean ± SE ($n = 3$).

3.3. *Inhibition of Ca^{2+} channel blocker*

Nilvadipine (Fujisawa Pharmaceutical Co., Ltd), a derivative of nifedipine, is a new Ca^{2+} channel blocker [9]. In the presence of 10^{-6} M nilvadipine, ET and VIC-induced contractions of guinea pig ilea were attenuated to a level of about 5%, as shown in fig.4.

4. DISCUSSION

VIC was found to have a much stronger contractile activity in mouse and guinea pig ileum than ET, but VIC was found to be a less potent vasoconstrictor in porcine coronary artery than ET. In addition, a transcript of this peptide gene was not detectable in endothelial cells, but was detected in the mouse intestine (Saida, K., Mitsui, Y. and Ishida, N., unpublished). Thus, this novel peptide might be classified as a gastrointestinal hormone. Then we propose designating it, 'VIC', i.e., vasoactive intestinal contractor. The fact that VIC and ET had differential contractile activities on rodent ileum and porcine coronary artery, suggests a possibility that minor difference in their receptors may exist in ileum and in vascular smooth muscle cells. However, we have previously shown that a transcript of ET is expressed in endothelial cells, but not in the intestine, while a transcript of VIC is expressed in the intestine, but not in endothelial cells (unpublished), prompting a hypothesis that the biological role of VIC and ET are distinguished by tissue-specific transcription of a ligand gene in vivo.

An inhibition experiment in the presence of a Ca^{2+} channel blocker (nilvadipine [9]) suggests that an influx of extracellular Ca^{2+} is required for the ileum contraction effect of VIC and ET.

The question as to whether VIC acts on a unique receptor in the intestine which is distinct from the ET receptor in vascular smooth muscle cells remains to be clarified. VIC's physiological roles in the gastrointestinal tract and other organs, as well as in the central and peripheral nervous systems, also remain to be determined.

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