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Short communication

Dual effect of [D-Pen^2, D-Pen^5]enkephalin on ion transport in guinea pig colon

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Abstract

Effects of the δ-opioid receptor ligand, [D-Pen^2, D-Pen^5]enkephalin (DPDPE) on basal and endothelin-1-induced ion secretion in guinea pig colon were investigated. Muscle-stripped segments of guinea pig colon were mounted in Ussing flux chambers and changes in the short-circuit current (I_{sc}) were monitored continuously. DPDPE significantly reduced baseline I_{sc} at a low dose, 1 nM; however DPDPE increased I_{sc} at 10 and 100 μM. Endothelin-1 stimulated ion secretion that was unaltered in tissues pretreated with DPDPE. In guinea pig colon, δ-opioid receptor activation evoked both a proabsorptive and prosecretory response.

Keywords: δ-Opioid receptor; Short-circuit current; Endothelin-1; Intestine, large

1. Introduction

Opioid receptors have been identified in the gastrointestinal tract of various species. In the guinea pig intestine, δ-opioid receptors are present in neurons of the myenteric plexus and submucous plexuses (Kromer and Beubler, 1993; Dockray, 1994) and on epithelial cells (Lang et al., 1996).

Several functional studies have shown that in the small intestine, δ-opioid receptor agonists are proabsorptive. This was first demonstrated by Kachur et al (1980) who showed that the δ-opioid receptor agonist, [D-Ala^2-D-Leu^5]enkephalin reduced the short-circuit current (I_{sc}) in guinea pig ileum, in a dose-dependent fashion. Similarly, Dobbins et al. (1980) showed that in rabbit ileum, the endogenous delta ligands, [D-Ala^2-Met^5]enkephalin, [Leu] enkephalin and [Met]-enkephalin significantly reduced the I_{sc}. Additionally, the proabsorptive effects of these peptides were partially mediated by inhibition of enteric secretomotor neurons. In a more recent study, Fox-Threlkeld et al. (1994) also reported that [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and Met-enkephalin evoked ion absorption in canine ileum by inhibiting the release of the neurotransmitter, vasoactive intestinal polypeptide (VIP) from enteric neurons.

Although several studies have demonstrated the proabsorptive effects of δ-opioid receptor ligands in the small intestine of various species, the effect of δ-opioid receptor activation on ion transport in the colon is not well understood. The focus of this study was to investigate the effect of the selective δ-opioid receptor agonist, DPDPE on basal and endothelin-1-induced ion secretion in guinea pig colon. Endothelin-1 is a potent secretagogue in guinea pig colon (Reddix et al., 1998) and has been implicated in the mechanisms underlying secretory diarrhea associated with inflammatory bowel disease, more specifically colitis and diabetes (Takashi et al., 1991; Murch et al., 1992). The anti-diarrheal agent, loperamide has been effective in the treatment of mild to moderate diarrhea. The proabsorptive effects of loperamide are mediated by activation of both μ-opioid receptors on smooth muscle and δ-opioid receptors on submucous secretomotor neurons and epithelial cells. This study focuses only on the proabsorptive effects mediated by the activation of δ-opioid receptors. Since endothelin-1 is a potent secretagogue and involved in the mechanisms underlying inflammatory bowel disease, this study was designed to determine whether the proabsorptive effect of loperamide was partially mediated by the inhibitory effect of δ-opioid receptor activation on endothelin-1-induced secretion.

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2. Materials and methods

2.1. Tissue preparation

Male albino guinea pigs (Harlan Sprague–Dawley, Indianapolis, IN) weighing 350–500 g were housed in metal cages with food and water ad libitum. The animals were stunned and exsanguinated. This method of euthanasia has been approved by the LSU Institutional Animal Care Committee and complies with federal regulations. Segments of distal colon were removed and opened along the mesenteric border. The intraluminal contents were removed and the segments were pinned with the mucosal side down onto a Sylgard-coated petri dish. Tissues were perfused with a chilled Krebs–Ringer solution (in mM): 120 NaCl, 6 KCl, 1.2 $MgCl_2$, 6 H_2O, 1.3 $NaH_2PO_4H_2$, 14.4 $NaHCO_3$, 2.5 $CaCl_2$, 12.5 glucose. The longitudinal and circular muscle layers along with the myenteric plexus were removed by blunt dissection leaving the submucosa/mucosa intact.

2.2. Short-circuit current (I_{sc}) measurements

Muscle-stripped-colonic segments were divided into 2–3 cm sheets and mounted in Ussing flux chambers with an area of 0.785 cm^2. Three to four adjacent sheets were obtained from the most distal portion of the colon from each animal. Tissues were bathed in a Krebs–Ringer solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. The chambers were designed with ports for Ringer-agar bridges and calomel half-cells for measurement of transmural potential difference. Throughout the experiment, tissues were short-circuited with a voltage clamp apparatus (Physiological Instruments, California) to abolish changes in potential difference. The I_{sc} served as an index of electrogenic ion transport. It was measured in microampere and normalized to the tissue surface area (cm^2). Prior to the experimental session, all tissues were paired according to the baseline I_{sc}, G_t and potential difference. To examine the proabsorptive effects of DPDPE, tissues exposed to increasing concentrations (1 nM to 100 μM) of DPDPE added to the serosal compartment at 10-min intervals. This concentration range spans from a physiological concentration to a supermaximal dose. A non-cumulative dose–response relationship was determined by adding each dose to separate tissues. DPDPE-induced changes in I_{sc} were calculated as the baseline I_{sc} minus DPDPE-induced changes in I_{sc} ($\mu A/cm^2$). In a separate study, tissues were pretreated with vehicle, 0.1-nM, 1-, 10- or 100-μM DPDPE 10 min prior to serosal addition of 50-nM endothelin-1 to the bathing solution. We have previously shown that 50-nM endothelin-1 evokes a secretory response that is reproducible (Reddix et al, 1998). The peak endothelin-1-induced secretory response was recorded in the absence and presence of DPDPE over the concentration range of 1 nM–100 μM.

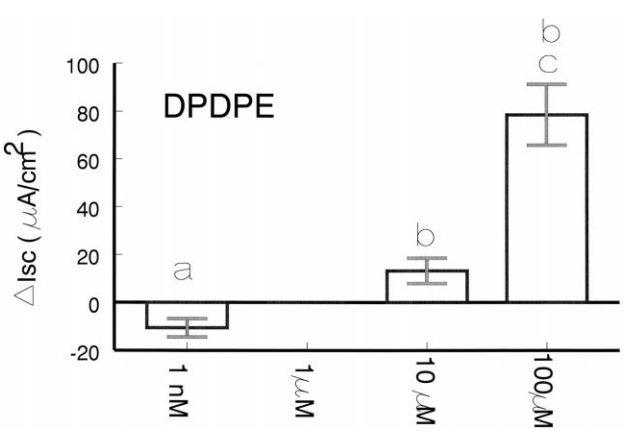

Fig. 1. Effect of DPDPE on baseline short-circuit current (I_{sc}; $\mu A/cm^2$) over the concentration range 1 nM to 100 μM in muscle-stripped segments of guinea pig colon. Vehicle (Krebs–Ringer buffer) had no effect on baseline I_{sc} (data not shown). Values are presented as mean ± S.E.M. $n = 4–5$ tissues from four guinea pigs. (a) Significantly less than zero; (b) significantly greater than zero; (c) significantly greater than 10-μM DPDPE. Statistical significance was examined at $p < 0.05$.

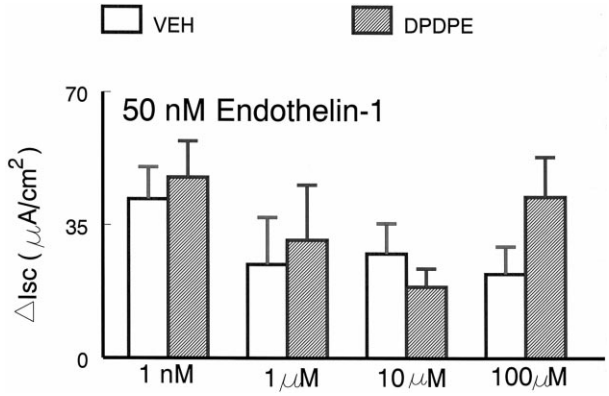

Fig. 2. Effect of DPDPE, 1 nM, 1, 10 and 100 μM on endothelin-1 (ET-1; 50 nM)-induced ion secretion. Vehicle (Krebs–Ringer buffer) had no effect on baseline I_{sc} (data not shown). ET-1 + Vehicle, Open squares □ and ET-1 + DPDPE, filled squares ■. Values are presented as mean ± S.E.M.; $n = 4–5$ tissues from four guinea pigs.

2.3. Chemicals

Endothelin-1 and DPDPE were purchased from Peninsula laboratories (Belmont, CA). Both peptides were dissolved in Krebs–Ringers buffer without glucose.

2.4. Statistical analysis

All data were presented as means ± standard error. A Student's *t*-test or a One-way analysis of variance was used to determine the statistical significance between control and experimental groups. A probability value of 0.05 or less was considered statistically significant.

3. Results

The results showed that the vehicle (Krebs–Ringer) had no effect on baseline I_{sc}. However, DPDPE reduced the I_{sc} at the low concentration, 1 nM (Fig. 1.). It had no effect at 1 μM and DPDPE stimulated ion secretion at 10 and 100 μM (Fig. 1.). As illustrated in Fig. 2, endothelin-1 (50 nM) increased baseline I_{sc} in all tissues. Pretreatment with 1 nM, 1-, 10- or 100-μM DPDPE had no effect on endothelin-1-induced secretion.

4. Discussion

Our results showed that the selective δ-opioid receptor agonist, DPDPE exhibited a dual effect on ion transport in guinea pig colon. At a low dose, 1 nM, DPDPE reduced ion transport. However, DPDPE stimulated ion secretion at the high concentrations, 10 and 100 μM. The endothelin-1-induced secretory response was unaltered by DPDPE at all concentrations.

A dual effect of opioid analogs on ion transport has been demonstrated, previously. Kromer and Beubler (1993) demonstrated that opioids may produce prosecretory or proabsorptive effects on prostaglandin plus theophylline stimulated secretion, depending on the specific opioid receptors activated (Kromer and Beubler, 1993). In their study, the μ-opioid receptor antagonist, CTOP-NH_2 and the κ-opioid receptor agonist, U69593 produced a proabsorptive response. Kromer (1995) more recently showed that at low (nM) concentrations, loperamide produced a prosecretory effect at μ-opioid receptors, whereas at higher (μM) concentrations, loperamide produced an ion absorbing response, which was not due to activation of opioid receptors (Kromer, 1995). This suggests that the prosecretory effects may be mediated by the activation of δ-opioid receptors as indicated by our results.

One possible explanation for the enhanced secretory effect that we observed, involves the role of G-proteins. The δ-opioid receptor is linked to the pertussis toxin-sensitive $G_{i/o}$ protein (Polastron and Jauzac, 1994). Activation of the δ-opioid receptor inhibits the stimulation of adenylate cyclase (a G_s protein mediated event) by the G_i protein, which blocks cyclic AMP (cAMP) production (Polastron and Jauzac, 1994). Cyclic AMP is a key intracellular messenger that mediates VIP, cholera toxin and prostaglandin-induced intestinal secretion (Cooke and Reddix, 1994). It seems possible that a higher concentrations of DPDPE, the secretory effect may involve non-specific activation of Gs-linked receptors.

We recently reported that endothelin-1 dose-dependently increased chloride secretion in muscle-stripped segments of guinea pig colon (Reddix et al., 1998). The endothelin-1 response was mediated by the activation of enteric secretomotor neurons and the release of prostaglandin E_2 (Reddix et al., 1998). In addition, the endothelin-1 response involved the activation of endothelin A receptors. It has been shown clinically, that endothelin-1 levels are elevated in inflammatory bowel disease and diabetes. Additionally, endothelin-1 is a potent secretagogue and its effects are partially mediated by prostaglandins, which have been shown to induce secretion in part by a cAMP-dependent mechanism. We were interested in determining whether the δ-opioid receptor analog, DPDPE would be effective in attenuating the endothelin-1-induced secretory response. Our results showed that DPDPE had no effect on endothelin-1-induced secretion. An explanation for this result may be that, the endothelin-1-induced secretory effect is mediated more by increased secretomotor neural activity, which does not involve the direct activation of G_s protein at 50 nM. Although, endothelin-1 evokes the release of prostaglandins whose secretory effect is partially mediated by cAMP, a reduction in this component may be masked by other secretory pathways activated by endothelin-1 and prostaglandins.

In conclusion, the δ-opioid receptor plays a dual role in the regulation of ion transport across guinea pig colonic epithelia. At low concentrations, δ-opioid analogs promote absorption; however, at high concentrations, they may stimulate ion secretion. Hence, caution should be exercised when using anti-diarrheal preparations such as loperamide whose mechanism of action partially involves the activation of δ-opioid receptors and/or specific δ-opioid receptor analogs. These agents may exacerbate the diarrheal state.

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