ELSEVIER

European Journal of Pharmacology 451 (2002) 185–190

www.elsevier.com/locate/ejphar

Active bicarbonate-dependent secretion evoked by 5-hydroxytryptamine in porcine ileal mucosa is mediated by opioid-sensitive enteric neurons

Benedict T. Green, David R. Brown *

Department of Veterinary PathoBiology, College of Veterinary Medicine, University of Minnesota, 1988 Fitch Avenue, St. Paul, MN 55108-6010, USA

Received 6 May 2002; accepted 2 July 2002

Abstract

5-Hydroxytryptamine (5-HT) mediates intestinal hypersecretion associated with infection and inflammation. We tested the hypothesis that 5-HT-induced anion secretion is mediated by an opioid-sensitive enteric neural circuit. 5-HT, at a contraluminal concentration of 10 μM, increased short-circuit current by 58 ± 7 μA/cm^2 in sheets of porcine ileal mucosa with attached inner submucosal plexus. Responses to 5-HT were inhibited by saxitoxin or indomethacin, and reduced in tissues bathed in Cl^- - or HCO_3^- -deficient media. 5-HT action was attenuated by saxitoxin in tissues bathed in Cl^- -free media, but not HCO_3-free media. The δ-opioid receptor agonist [D-Pen^{2,5}]enkephalin (0.1 μM) blunted the 5-HT change in short-circuit current by a mechanism sensitive to the δ-opioid receptor antagonist naltrindole. The inhibitory actions of [D-Pen^{2,5}]enkephalin and saxitoxin were not additive. These results suggest that 5-HT stimulates HCO_3^- -dependent ion transport through a mechanism involving prostanoids and an enteric neural pathway modulated by opioids.

Keywords: Bicarbonate-dependent secretion; Cannabinoid; Enkephalin; Inflammatory mediator; Prostanoid

1. Introduction

The intestinal mucosa represents an extensive surface area that is in contact with the external environment and must simultaneously absorb dietary nutrients, ions and water; coexist with commensal enteric microflora; and exclude luminal pathogens and other noxious agents. It has therefore evolved a diverse array of specific and non-specific protective mechanisms against infection. The active secretion of chloride and bicarbonate ions by the intestinal epithelium is an example of one such host defense process that is under neuroimmune modulation (Perdue and McKay, 1994). The serosa-to-lumen anion flux creates an osmotic gradient that promotes the passive transport of water into the lumen. This net luminal flux of ions with secondary water movement is thought to dilute luminal pathogens and facilitate the transfer of protective substances such as mucus, antimicrobial peptides and secretory immunoglobulin A to the mucosal surface (Hecht, 1999).

5-Hydroxytryptamine (5-HT) is a prominent neuroimmunomodulatory substance that is present in enterochromaffin cells, enteric neurons and, in the case of rodents, mucosal mast cells of the intestine (Gershon, 1999). Its receptors appear to be present in enteric neural pathways that trigger mucosal protective mechanisms, such as active anion secretion (Brown, 1996). Indeed, 5-HT appears to mediate neurogenic secretion induced by enteropathogens such as *Vibrio cholerae* and *Salmonella typhimurium* (Lundgren, 1998). It is also involved in neurogenic secretion evoked by mechanical stimulation of the intestinal mucosa (Kellum et al., 1999). Natural and synthetic opioids can arrest diarrhea and produce constipation, actions which have been attributed in part to their stimulation of inhibitory δ-opioid receptors expressed on enteric submucosal neurons, which are linked to mucosal secretion (De Luca and Coupar, 1996). In the rat intestine, the antisecretory actions of morphine and other opioid antidiarrheal drugs are dependent upon enteric 5-HT neural pathways (Coupar and Taylor, 1987; De Luca and Coupar, 1993).

In the present investigation, we addressed the hypothesis that 5-HT-induced anion secretion is mediated by opioid-sensitive enteric neural circuits. The porcine ileum was used as a biological model because of its homology with the

* Corresponding author. Tel.: +1-612-624-0713; fax: +1-612-625-0204.
E-mail address: brown013@tc.umn.edu (D.R. Brown).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02249-5

human gut (Swindle and Smith, 1998). The receptors mediating the actions of 5-HT and opioids on active anion secretion have been characterized previously in isolated mucosa–submucosa preparations of porcine small intestine (Hansen and Skadhauge, 1997; Poonyachoti et al., 2001). Furthermore, δ-opioid receptor immunoreactivity has been localized in submucosal neurons and fibers of porcine ileum (Poonyachoti et al., 2001). Cannabinoids have antipropulsive actions in the intestine that are similar to opioids, but their ability to alter intestinal secretion has not been clearly defined (Izzo et al., 2001). As cannabinoid CB_1 receptor immunoreactivity has recently been detected in the porcine enteric nervous system, it was of interest to compare the effects of the potent cannabinoid agonist HU-210 with those of the selective δ-opioid receptor agonist DPDPE on 5-HT-induced ion transport in the porcine intestinal mucosa (Kulkarni-Narla and Brown, 2000).

2. Materials and methods

2.1. Animals

Intestinal tissues were obtained from Yorkshire pigs (6–10 weeks of age; 10–18 kg body weight) of each sex which were not fasted before sacrifice. Animals were sedated with an intramuscular injection of tiletamine hydrochloride-zolazepam (Telazol®; 8 mg/kg, Fort Dodge Laboratories, Fort Dodge, IA), in combination with xylazine (8 mg/kg). The animals were subsequently euthanized by barbiturate overdose in accordance with approved University of Minnesota Animal Care Committee protocols. A midline laparotomy was performed to expose the intestine and a portion of the ileum, identified by its attachment to the ileo-cecal ligament, was removed and placed in an oxygenated organ preservation solution (composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 0.5; $NaHCO_3$, 25; NaH_2PO_4, 1.0; and D-glucose, 11; pH 7.4).

2.2. Chemicals and drugs

5-Hydroxytryptamine (5-HT), atropine, indomethacin, bumetanide and carbamylcholine chloride were obtained from Sigma (St. Louis, MO). Saxitoxin and naltrindole were purchased from Research Biochemicals (Natick, MA). [D-Pen^{2,5}]enkephalin (DPDPE) was purchased from Peninsula Laboratories (Belmont, CA). HU-210 (6*aR*)-*trans*-3-(1,1-dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol) was purchased from Tocris Cookson (Ballwin, MO). HU-210 and indomethacin were solubilized in dimethylsulfoxide (DMSO). DPDPE was solubilized in 0.01 M acetic acid with 0.1% bovine serum albumin, aliquoted at stock concentrations of 100 μM, and stored until use at −65 °C. All other drugs and reagents were dissolved in distilled water.

2.3. Measurement of transepithelial ion transport

Ileal segments were stripped of their overlying smooth muscle coats and sheets of mucosa–submucosa were mounted between two Lucite half-chambers (Jim's Instrument Manufacturing, Iowa City, IA) having a flux area of 2 cm^2. Mucosal sheets were bathed on both sides with a physiological salt solution that approximated the composition of the porcine extracellular fluid (composition in mM: NaCl, 130; KCl, 6; $CaCl_2$, 3; $MgCl_2$, 0.7; $NaHCO_3$, 20; NaH_2PO_4, 0.29; and Na_2HPO_4, 1.3) at pH 7.4 and gassed continuously with 5% CO_2 in O_2 at 39 °C (porcine core temperature). In anion substitution experiments, gluconic acid replaced chloride ion and HEPES was substituted for bicarbonate ion at equimolar concentrations. Tissues in the bicarbonate substitution experiments were gassed with O_2. D-Glucose and mannitol (10 mM) were added to the contraluminal and luminal bathing media, respectively.

The short-circuit current (I_{sc}, in μA/cm^2) across each mucosa–submucosal sheet, a measure of net, active ion transport, was monitored continuously by an automatic voltage clamp apparatus (Model TR100, JWT Engineering, Overland Park, KS) with the serosal side as reference. Experiments were initiated after the basal I_{sc} had stabilized (within 25–35 min). Changes in current response to the delivery of a 5-mV pulse were periodically measured during each experiment in order to calculate tissue conductance (G_t in mS/cm^2) according to Ohm's law. Peak changes in I_{sc} were measured immediately before and after drug administration. At the end of each experiment when the I_{sc} returned to baseline values, mucosal I_{sc} responses to 10 μM carbachol (contraluminal addition) and 10 mM glucose (luminal addition) were measured in each tissue to assess tissue viability. Data from tissues that did not respond to both carbachol and glucose with I_{sc} elevations were omitted from data analysis.

5-HT was added to the contraluminal bathing medium to achieve a final bath concentration of 10 μM, which approximates the 50% effective concentration to produce active ion secretion in porcine jejunum determined previously (Hansen et al., 1994b). In some experiments, blockers were added to the contraluminal bathing medium 10 min prior to 5-HT addition. Some tissues were pretreated contraluminally with 0.1 μM DPDPE or 1 μM HU-210 for 10 min prior to 5-HT addition; in some experiments with DPDPE, either saxitoxin (0.1 μM) or the δ-opioid receptor antagonist naltrindole (1 μM) was added to the contraluminal bathing medium 5 min prior to DPDPE addition.

2.4. Data analysis

Data are expressed as mean I_{sc} or G_t under baseline conditions, or as mean peak changes in I_{sc} occurring in response to 5-HT. Statistical analyses of data were performed using the PRISM computer software program (version 2.0; GraphPad Software, San Diego, CA). Comparisons

between a control mean and a single treatment mean were made by unpaired *t*-tests. Comparisons of a control mean with multiple treatment means were made by one-way analysis of variance followed by Dunnett's test. In all cases, the limit for statistical significance was set at $P < 0.05$.

3. Results

3.1. Mediators of 5-HT action

Baseline I_{sc} and G_t in isolated sheets of ileal mucosa–submucosa averaged 5 ± 3 μA/cm^2 and 28 ± 1 mS/cm^2 ($n = 189$ tissues from 31 pigs). At a contraluminal concentration of 10 μM, 5-HT produced a monophasic rise in I_{sc} that attained a peak elevation of 58 ± 7 μA/cm^2 relative to mean baseline values ($n = 61$ tissues from 31 pigs). The 5-HT-induced increase in I_{sc} was 129% of that produced by 10 μM carbachol (ΔI_{sc} produced by carbachol $= 45 \pm 4$ μA/cm^2, $n = 61$ tissues from 31 pigs). The mean duration of the I_{sc} elevation induced by 5-HT was 8.6 ± 0.5 min (8 tissues from 4 pigs). Tissue conductance increased transiently by 21 ± 8 mS/cm^2 when determined at the time of peak I_{sc} change produced by 5-HT ($n = 61$ tissues from 31 pigs).

To verify that the action of 5-HT was mediated by enteric neurons, mucosal I_{sc} responses to 5-HT were examined in 14 tissues pretreated with 0.1 μM saxitoxin, a neuronal Na^+ channel blocker. The neurotoxin did not significantly alter baseline I_{sc} or G_t. In the presence of saxitoxin, I_{sc} elevations produced by 5-HT were decreased to 36 ± 10% of those in saxitoxin untreated tissues (Fig. 1).

To determine if the activation of muscarinic acetylcholine receptors was involved in 5-HT action, 10 tissues were pretreated with the muscarinic acetylcholine antagonist, atropine. At a contraluminal concentration of 0.1 μM, atropine did not significantly alter baseline I_{sc} or G_t, and mucosal I_{sc} responses to 5-HT were not significantly altered in atropine-treated tissues (Fig. 1).

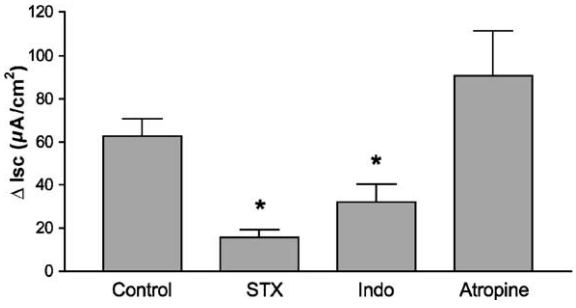

Fig. 1. Inhibitory effects of saxitoxin (STX), indomethacin (Indo) or atropine on subsequent mucosal I_{sc} responses to 5-hydroxytryptamine (5-HT) in the porcine ileal mucosa. All drugs were administered to the contraluminal aspect of tissues at a concentration of 0.1 (saxitoxin, atropine) or 10 μM (5-HT, indomethacin). Bars represent the mean ± S.E. changes in I_{sc} produced by 5-HT in 47 control tissues (from 20 pigs), 14 tissues (11 pigs) pretreated with saxitoxin, 20 tissues (9 pigs) pretreated with indomethacin, and 9 tissues (7 pigs) pretreated with atropine. * $P < 0.05$ vs. control mean, Dunnett's test.

Because 5-HT action may be dependent upon the formation of eicosanoids, mucosal responses to 5-HT were measured in 17 tissues pretreated with indomethacin, a cyclooxygenase inhibitor. At a contraluminal concentration of 10 μM, indomethacin did not significantly alter baseline I_{sc} or G_t. However, it significantly decreased 5-HT-induced I_{sc} responses to 45% of peak I_{sc} elevations in untreated tissues (Fig. 1). DMSO was used to solubilize indomethacin and bumetanide, and the effects of this solvent on 5-HT-induced I_{sc} elevations were examined as well. At a contraluminal concentration of 0.1% v/v, it did not significantly change baseline I_{sc} or G_t, or alter significantly mucosal responses to 10 μM 5-HT (mean ΔI_{sc} after 5-HT in DMSO-treated tissues $= 79 \pm 18$ μA/cm^2; $P > 0.05$ vs. response in DMSO untreated tissues, *t*-test; $n = 12$ tissues from 7 pigs).

3.2. Effects of 5-HT on neurogenic anion-dependent transport

The $Na^+/K^+/Cl^-$ cotransport blocker bumetanide, an inhibitor of one Cl^- entry pathway in intestinal epithelial cells that is of importance in active anion secretion, did not significantly change baseline I_{sc} or G_t after its contraluminal addition at 10 μM. However, it halved peak I_{sc} elevations occurring in response to contraluminal 5-HT (ΔI_{sc} produced by 10 μM 5-HT in the absence and presence of bumetanide $= 75 \pm 12$ and 44 ± 6 μA/cm^2; $P < 0.05$, unpaired *t*-test; $n = 18$ and 22 tissues from 8 pigs, respectively).

Anion substitution experiments were undertaken to assess the dependency of the I_{sc} response to 5-HT on extracellular Cl^- and HCO_3^-. Gluconate was substituted for the Cl^- ions in bathing media to examine the role of HCO_3^- ions in 5-HT action; HEPES was substituted for HCO_3^- ions in HCO_3^--deficient/Cl^--replete media. Removal of Cl^- or HCO_3^- ions reduced baseline I_{sc} to equivalent levels (mean I_{sc} in anion-replete, Cl^--deficient and HCO_3^--deficient conditions was 16 ± 7, 5 ± 5 and 4 ± 4 μA/cm^2, respectively, $n = 21–93$ tissues from 16 to 24 pigs). Tissue conductance was decreased in either Cl^-- or HCO_3^--free media as well (mean G_t in anion-replete, Cl^--deficient and HCO_3^--deficient conditions was 29 ± 3, 16 ± 1 and 24 ± 1 mS/cm^2, respectively; $P < 0.05$ for each anion-deficient condition vs. the anion-replete condition, Dunnett's test, $n = 21–93$ tissues from 16 to 24 pigs).

The peak elevations in I_{sc} produced by 5-HT in tissues bathed in HCO_3^--deficient and Cl^--deficient media were significantly decreased from those in tissues bathed in anion-replete media (mean ΔI_{sc} produced by 10 μM 5-HT in anion-replete, Cl^--deficient media and HCO_3^--deficient media $= 60 \pm 11$, 21 ± 4 and 21 ± 2 μA/cm^2; $P < 0.05$, Dunnett's test, $n = 24–34$ tissues from 9 to 24 pigs, respectively).

In tissues bathed in Cl^--deficient media, saxitoxin significantly reduced 5-HT-induced elevations in I_{sc} (Fig. 2A). In contrast, I_{sc} responses to 5-HT in tissues bathed in HCO_3^--deficient media were relatively resistant to the neurotoxin (Fig. 2B). Indomethacin significantly reduced I_{sc} responses to 5-HT in tissues bathed in media deficient in Cl^- ion (Fig. 2A).

Mucosal I_{sc} responses to 5-HT in tissues bathed in media deficient in either anion were not significantly altered by bumetanide (Fig. 2).

3.3. Effects of opioid and cannabinoid agonists on 5-HT-stimulated ion transport

The δ-opioid receptor agonist DPDPE (0.1 μM, contraluminal addition) did not produce significant changes in baseline I_{sc} or G_t. However, it blunted the effect of 5-HT on I_{sc} (Fig. 3). The competitive δ-opioid receptor antagonist naltrindole prevented the inhibitory action of DPDPE (Fig. 3). Moreover, tissues to which naltrindole and DPDPE were added displayed augmented I_{sc} responses to 5-HT (Fig. 3). In tissues pretreated with saxitoxin, DPDPE produced no additional decrease in 5-HT-induced I_{sc} elevations from baseline values (13 ± 2 μA/cm^2; 19 tissues from 6 pigs).

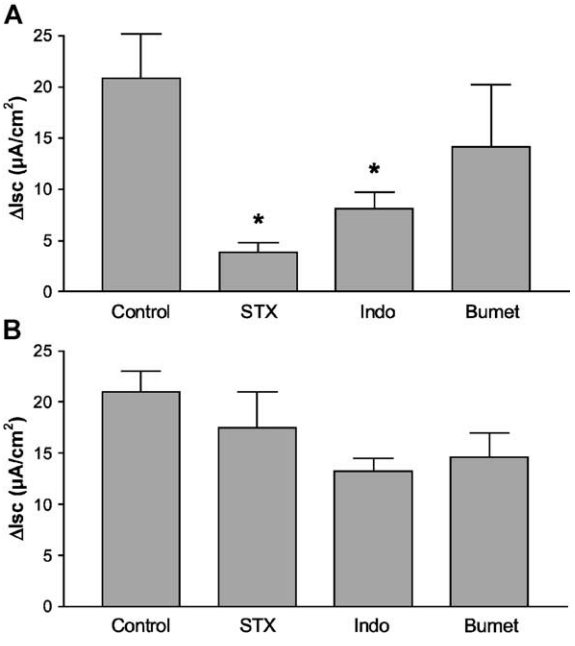

Fig. 2. Dependence of 5-HT-induced I_{sc} elevations on extracellular anions. Responses to 5-HT (10 μM) were measured in mucosal sheets bathed in (A) Cl^--deficient media or (B) HCO_3^--deficient media in the absence (control) and presence of contraluminal saxitoxin (STX, 0.1 μM), indomethacin (Indo, 10 μM) or bumetanide (Bumet, 10 μM). Bars over "control" represent the mean (± S.E.) changes in I_{sc} produced by 5-HT in 24 (from 16 pigs) or 34 (19 pigs) tissues serving as controls that were bathed in Cl^-- or HCO_3^--deficient media, respectively. Mean (± S.E.) changes in I_{sc} produced by 5-HT are also represented for 6–9 tissues (5 pigs) pretreated with saxitoxin, 17–22 tissues (9–11 pigs) pretreated with indomethacin, and 7–22 tissues (6–13 pigs) pretreated with bumetanide that were bathed in media deficient in either anion. * $P < 0.05$ vs. control mean, Dunnett's test.

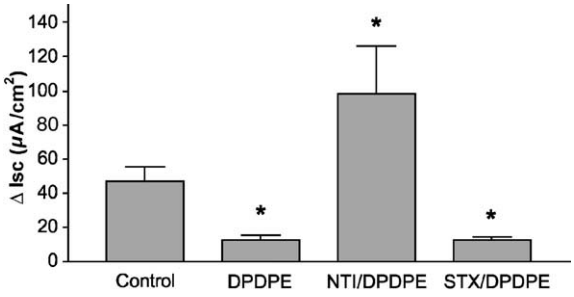

Fig. 3. Inhibitory action of the δ-opioid receptor agonist [D-Pen^{2,5}]enkephalin (DPDPE) on 5-HT-induced I_{sc} elevations in porcine ileal mucosa. Responses to contraluminal 5-HT (10 μM) were measured in mucosal sheets in the absence (control) or presence (DPDPE) of the opioid agonist at a contraluminal concentration of 0.1 μM. In some tissues, either the δ-opioid receptor antagonist naltrindole (NTI) or saxitoxin (STX) was present at respective contraluminal concentrations of 1 and 0.1 μM prior to DPDPE addition. Bars represent the mean ± S.E. changes in I_{sc} produced by 5-HT measured in 13 control tissues (from 7 pigs), 20 tissues (7 pigs) pretreated with DPDPE, 8 tissues (4 pigs) pretreated with NTI and DPDPE, and 19 tissues (6 pigs) pretreated with STX and DPDPE. * $P < 0.05$ vs. control condition, Dunnett's test.

At a relatively high contraluminal concentration of 1 μM, the potent cannabinoid agonist HU-210 did not produce significant changes in baseline I_{sc} or G_t, and did not affect subsequent mucosal responses to 5-HT (ΔI_{sc} after 5-HT in HU-210-treated tissues = 50 ± 15 μA/cm^2; 4 tissues from 4 pigs).

4. Discussion

In the porcine small intestine, 5-HT has been found previously to stimulate fluid accumulation in isolated intestinal loops and to increase I_{sc} and net ^{36}Cl secretory flux in mucosal sheets (Hansen et al., 1994a,b). These actions appear to be mediated by epithelial 5-HT_2 receptors and neuronal 5-HT_3 and 5-HT_4 receptors (Hansen et al., 1994c; Hansen and Skadhauge, 1997). Similarly, 5-HT_4 receptors may mediate I_{sc} elevations produced by 5-HT in the human ileum (Borman and Burleigh, 1993). In this investigation, peak I_{sc} elevations in mucosa–submucosa sheets from porcine ileum occurring after contraluminal administration of 5-HT were blunted by the neuronal conduction blocker saxitoxin, providing evidence that 5-HT action is mediated in part by enteric neurons. The neural pathway involved does not appear to contain muscarinic acetylcholine receptors because of the insensitivity of 5-HT action to atropine. Similar results were obtained in previous studies of the porcine jejunal mucosa (Hansen, 1994). In intestinal mucosal preparations from several species, prostanoids appear to play a role in the secretory effects of 5-HT. In human and porcine jejuna, for example, 5-HT increases the luminal release of prostaglandin E_2, and the cyclooxygenase inhibitor indomethacin reduces 5-HT-induced secretion

(Munck et al., 1988; Hansen and Skadhauge, 1997; Hansen et al., 1994b). In the present study, indomethacin similarly reduced the ileal I_{sc} response to 5-HT, indicating partial mediation of this effect by prostanoids. Although the mechanisms underlying 5-HT secretory effects are complex and 5-HT secretory efficacy may vary in magnitude along the length of the porcine intestine, enteric neurons and prostanoids appear to mediate 5-HT-induced mucosal transport in both the jejunum and ileum (Grøndahl et al., 1996a; Hansen and Skadhauge, 1997).

5-Hydroxytryptamine-induced elevations in ileal I_{sc}were due in part to active Cl^- secretion because they were sensitive to the loop diuretic bumetanide, which blocks Cl^- loading in enterocytes through the $Na^+/K^+/Cl^-$ co-transporter. This drug also reduced I_{sc} responses to 5-HT by a similar magnitude in porcine jejunum (Hansen, 1994). Bumetanide sensitivity provides indirect evidence that Cl^- secretion is a component of 5-HT action. Anion substitution experiments were undertaken to examine in further detail the anions contributing to this effect. The effects of 5-HT on I_{sc} were reduced by similar magnitudes in tissues bathed in media deficient in either Cl^- or HCO_3^- ions, indicating that they are dependent on both extracellular anions. Saxitoxin nearly abolished I_{sc} responses to 5-HT in tissues bathed in Cl^--deficient media. On the other hand, the effect of 5-HT in tissues bathed in HCO_3^--free media was relatively resistant to saxitoxin. These results suggest that 5-HT stimulates neurogenic HCO_3^--dependent ion transport, quite possibly active HCO_3^- secretion, in porcine ileum. Based on these data, we hypothesize that electrogenic HCO_3^- sec- retion underlies the neuronal component of 5-HT action and Cl^- secretion may be induced by 5-HT acting through a saxitoxin-resistant mechanism, possibly through a direct action on enterocytes. Prostanoids appear to modulate electrogenic ion transport induced by 5-HT that is predominately dependent upon extracellular HCO_3^- because indomethacin did not reduce 5-HT actions significantly in ileal mucosae bathed in HCO_3^--deficient media.

Enteric δ-like opioid receptors appear to modulate electrogenic ion transport evoked by transmural stimulation of submucosal neurons in the porcine ileum (Poonyachoti et al., 2001). Moreover, I_{sc} elevations produced by either trypsin, histamine, or an immediate hypersensitivity reaction to a food allergen in the porcine ileal mucosa are attenuated by the δ-opioid receptor agonist DPDPE (Green et al., 2000; Poonyachoti and Brown, 2001). The secretory effects of trypsin and histamine, which are mediated respectively by type 2 proteinase-activated receptors and H_1-histamine receptors, are similar to those of 5-HT in their sensitivity to saxitoxin and the loop diuretic furosemide. The I_{sc}-elevating action of trypsin, like that of 5-HT, is also sensitive to indomethacin. We tested the hypothesis that δ-opioid receptors are expressed in a common enteric neuronal circuit that mediates secretory responses to inflammatory mediators, including histamine, mast cell tryptase, kallidin and 5-HT. In support of this hypothesis, DPDPE markedly attenuated mucosal I_{sc} responses to 5-HT, and its effects were prevented by the selective δ-opioid receptor antagonist, naltrindole. DPDPE did not further inhibit 5-HT action in tissues pretreated with saxitoxin. Based on these results and our previous data, we postulate that the indomethacin-sensitive portion of HCO_3^--dependent, neurogenic ion transport stimulated by 5-HT is mediated through an opioid-sensitive enteric neural pathway. Cannabinoids, like opioids, decrease intestinal propulsion but their actions on mucosal ion transport have not been clearly defined (Izzo et al., 2001). Immunoreactivity for cannabinoid CB_1 receptors has been localized in myenteric and submucosal neurons in the porcine ileum, and immunorective fibers appear to innervate mucosal crypts (Kulkarni-Narla and Brown, 2000). However, unlike DPDPE, the potent cannabinoid agonist HU-210 did not alter mucosal I_{sc} responses to 5-HT. This is consistent with preliminary studies in our laboratory indicating that cannabinoids, in contrast to opioids, do not affect neurogenic ion transport evoked by transmural electrical stimulation in sheets of porcine ileal mucosa–submucosa (Poonyachoti et al., unpublished data).

The δ-opioid receptor agonist DPDPE, but not the cannabinoid receptor agonist HU-210, inhibited saxitoxin-sensitive (i.e. neurogenic) secretion induced by 5-HT in porcine small intestine. Intestinal secretion induced by *V. cholerae* enterotoxin or epithelial invasion of *S. typhimurium* is mediated in part by 5-HT and its receptors in the porcine enteric nervous system (Grøndahl et al., 1996b, 1998). By inhibiting secretory responses to 5-HT, opioids may impair an important aspect of mucosal defense against these and perhaps other enteropathogens.

Acknowledgements

The authors thank Dr. Scott M. O'Grady (Department of Animal Science-Physiology, University of Minnesota) for expert technical advice during the execution of this project. This study was funded in part by NIH/NIDA grant R01 DA-10200. B.T.G. was a predoctoral trainee supported by ADAMHA/NIDA Psychoneuroimmunology and Substance Abuse training grant T32 DA07239.

References

Borman, R.A., Burleigh, D.E., 1993. Evidence for the involvement of a 5-HT_4 receptor in the secretory response of human small intestine to 5-HT. Brit. J. Pharmacol. 110, 927–928.

Brown, D.R., 1996. Mucosal protection through active intestinal secretion: neural and paracrine modulation by 5-hydroxytryptamine. Behav. Brain Res. 73, 193–197.

Coupar, I.M., Taylor, D.A., 1987. Evidence for tryptaminergic and noradrenergic involvement in the antisecretory action of morphine in the rat jejunum. J. Pharm. Pharmacol. 39, 363–369.

De Luca, A., Coupar, I.M., 1993. Difenoxin and loperamide: studies on

possible mechanisms of intestinal antisecretory action. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 231–237.

De Luca, A., Coupar, I.M., 1996. Insights into opioid action in the intestinal tract. Pharmacol. Ther. 69, 103–115.

Gershon, M.D., 1999. Roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment. Pharmacol. Ther. 13 (Suppl. 2), 15–30.

Green, B.T., Bunnett, N.W., Kulkarni-Narla, A., Steinhoff, M., Brown, D.R., 2000. Intestinal type 2 proteinase-activated receptors: expression in opioid-sensitive secretomotor neural circuits that mediate epithelial ion transport. J. Pharmacol. Exp. Ther. 295, 410–416.

Grøndahl, M.L, Hansen, M.B., Larsen, I.E., Skadhauge, E., 1996a. Age and segmental differences in 5-hydroxytryptamine-induced hypersecretion in the pig small intestine. J. Comp. Physiol., B 166, 21–29.

Grøndahl, M.L., Jensen, G.M., Skadhauge, E., Hansen, M.B., 1996b. Effect of 5-HT_2 and 5-HT_3 receptor antagonists on cholera toxin-induced fluid hypersecretion in the pig jejunum. Zentralbl. Veterinarmed., Reihe A 43, 543–552.

Grøndahl, M.L., Jensen, G.M., Nielsen, C.G., Skadhauge, E., Olsen, J.E., Hansen, M.B., 1998. Secretory pathways in *Salmonella typhimurium*-induced fluid accumulation in the porcine small intestine. J. Med. Microbiol. 47, 151–157.

Hansen, M.B., 1994. ICS 205–930 reduces 5-methoxytryptamine-induced short-circuit current in stripped pig jejunum. Can. J. Physiol. Pharm. 72, 227–232.

Hansen, M.B., Skadhauge, E., 1997. Signal transduction pathways for serotonin as an intestinal secretagogue. Comp. Biochem. Physiol. 118A, 283–290.

Hansen, M.B., Thørboll, J.E., Beubler, E., Skadhauge, E., 1994a. Effect of indomethacin, renzapride, methysergide, ketanserin, granisetron and citalopram on serotonin-induced fluid accumulation in pig jejunum. Physiol. Res. 43, 83–93.

Hansen, M.B., Thørboll, J.E., Christensen, P., Bindslev, N., Skadhauge, E., 1994b. Serotonin-induced short-circuit current in pig jejunum. Zentralbl. Veterinarmed., Reihe A 41, 110–120.

Hansen, M.B., Thørboll, J.E., Skadhauge, E., 1994c. 5-Hydroxytryptamine 2 and 5-hydroxytryptamine 3 receptors mediate serotonin-induced short-circuit current in pig jejunum. J. Comp. Physiol., B 164, 343–348.

Hecht, G., 1999. Innate mechanisms of epithelial host defense: spotlight on intestine. Am. J. Physiol. 277, C351–C358.

Izzo, A.A., Mascolo, N., Capasso, F., 2001. The gastrointestinal pharmacology of cannabinoids. Curr. Opin. Pharmacol. 1, 597–603.

Kellum, J.M., Albuquerque, F.C., Stoner, M.C., Harris, R.P., 1999. Stroking human jejunal mucosa induces 5-HT release and Cl^- secretion via afferent neurons and 5-HT_4 receptors. Am. J. Physiol. 277, G515–G520.

Kulkarni-Narla, A., Brown, D.R., 2000. Localization of CB_1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. Cell Tissue Res. 302, 73–80.

Lundgren, O., 1998. 5-Hydroxytryptamine, enterotoxins, and intestinal fluid secretion. Gastroenterology 115, 1009–1012.

Munck, L.K., Mertz-Nielsen, A., Westh, H., Bukhave, K., Beubler, E., Rask-Madsen, J., 1988. Prostaglandin E_2 is a mediator of 5-hydroxytryptamine induced water and electrolyte secretion in the human jejunum. Gut 29, 1337–1341.

Perdue, M.H., McKay, D.M., 1994. Integrative immunophysiology in the intestinal mucosa. Am. J. Physiol. 267, G151–G165.

Poonyachoti, S., Brown, D.R., 2001. Delta-opioid receptors inhibit neurogenic intestinal secretion evoked by mast cell degranulation and type I hypersensitivity. J. Neuroimmunol. 112, 89–96.

Poonyachoti, S., Portoghese, P.S., Brown, D.R., 2001. Pharmacological evidence for a 7-benzylidenenaltrexone-preferring opioid receptor mediating the inhibitory actions of peptidic delta- and mu-opioid agonists on neurogenic ion transport in porcine ileal mucosa. J. Pharmacol. Exp. Ther. 297, 672–679.

Swindle, M.M., Smith, A.C., 1998. Comparative anatomy and physiology of the pig. Scand. J. Lab. Anim. Sci. 25 (Suppl. 1), 11–21.