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Evidence for down-regulation of neurogenic secretion in small intestinal epithelium from weaned piglets suffering from diarrhea ☆

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ABSTRACT

It is well known that active ion transport of the intestinal mucosa is regulated by the enteric nervous system (ENS) at least under physiological conditions. ENS control of the mucosa under pathophysiological situations such as diarrhea is less well studied. To address this question, we used a pig model of secretory diarrhea which is induced within hours in freshly weaned animals after oral treatment with enteropathogenic *Escherichia coli* Abbotstown (EcA). Two days after challenge jejunal segments were isolated and mucosa–submucosa preparations including neurons of the plexus submucosus were mounted in Ussing chambers. Net electrogenic transport of electrolytes was measured as short-circuit current (I_{sc}) in the absence and presence of pharmacological stimulation. Neurogenic secretion was measured as I_{sc} responses to electrical field stimulation. Basal I_{sc} values indicated higher chloride secretion in EcA animals as in control piglets. EFS induced secretion was only half as high in EcA animals as in controls indicating down-regulation of neurogenic secretory pathways. This assumption was supported by the finding that maximal stimulation of Cl^- secretion by forskolin and carbachol was nearly identical in both groups which means similar secretory capacities, but EFS induced I_{sc} responses were significantly lower in EcA animals after forskolin and carbachol pre-stimulation. The results suggest that pro-secretory ENS pathways may be down-regulated in situations when secretory processes are markedly stressed, i.e. in acute secretory diarrhea.

1. Introduction

The enteric nervous system (ENS) is one of the major components which modulate active transepithelial ion transport in small and large intestinal mucosa (Christofi, 2008; Hansen, 2003; Lundgren, 2002). At least under physiological conditions, this occurs via up- and down-regulation of pro-secretory, neurogenic pathways as for example shown in pig (Townsend et al., 2005; Brown & O'Grady, 1997). In the past, it has been shown that chemical or electrical stimulation of intrinsic enteric neurons in preparations of smooth-muscle-stripped mucosa of porcine jejunum mucosa with attached submucosa is associated with an increase of short-circuit current (I_{sc}), a measure of active chloride secretion (Townsend et al., 2005; Hildebrand & Brown, 1990). However, comparatively little is known about the ENS response in a situation when augmented fluid and electrolyte secretion is induced, i.e. by luminal secretagogues such as enterotoxigenic *Escherichia coli*. To address this question, we used a pig model which suffers from acute secretory diarrhea, which can be induced by treating the animals with the porcine *E. coli* Abbotstown (EcA, Schroeder et al., 2006). From that study contribution of the jejunum in maintaining diarrhea was concluded and recently it could be shown that the disposition for jejunal hypersecretion was more pronounced in freshly weaned piglets than in older animals (Leonhard-Marek et al., 2009). In the present study we aimed at studying the contribution of the ENS to jejunal chloride secretion as indicated by respective short-circuit currents under control and EcA infected conditions.

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2. Materials and methods

2.1. Animals

In experiment I (Exp. I), eight healthy male (German landrace × Pietrain) hybrid piglets with an age of 3 to 4 months (34.6 ± 0.6 kg body weight), fed with standard pig starter diet, were used. In experiment II (Exp. II), thirty-five weaned hybrids of mixed sex could be used for the study. The animals originated from five mother sows (1st litter 8 animals; 2nd 10, 3rd 6, 4th 6, and 5th 5) which were housed in the stables of the Department of Physiology for at least 4 weeks antepartum. Piglets were maintained suckling for 3 weeks and were given free access to additional food from day 7 after birth (Schroeder et al., 2006).

2.2. Challenge with E. coli Abbotstown

At day 21 after birth, piglets of Exp. 2 were weaned and randomly assigned to be challenged with the toxigenic *E. coli* Abbotstown (EcA, N = 23) or to be treated with a placebo (controls, N = 12). Inocula of EcA containing $1–2 \times 10^{10}$ cfu were administered to piglets via a stomach tube at 4 and 24 h after weaning (Schroeder et al., 2006). On a 3-rank scale (1: clean and dry, 2: moderately contaminated, and 3: extensively contaminated) most of the challenged piglets showed moderate fecal contamination of the region surrounding the anus. Similarly, fecal consistencies on a 4-rank scale of 1: unchanged, 2: soft, 3: semi-liquid and 4: watery feces looked soft to semi-liquid in the same animals. All control animals remained unaffected.

2.3. Intestinal tissues

Piglets of Exp. 1 or Exp. 2 (48 h after first EcA infection or placebo treatment) were euthanized by exsanguination following mechanical stunning. A 40 cm segment of distal jejunum was obtained, starting 50–60 cm oral from the ileo-colic orifice. By this procedure we obtained tissues in the same relative distance from the terminal ileum at the ileo-colic orifice and took into account the age dependent increase in jejunal length. The serosa and smooth-muscle layers of an excised tissue were removed and the remaining submucosa–mucosa sheet was mounted in Ussing chambers with a measuring area of 1 cm^2, equipped with aluminum foil for electrical field stimulation (EFS). The mucosal and serosal sides were bathed separately with 10 ml of modified Krebs–Henseleit buffer solutions (mucosal KH1; serosal KH2), both adjusted to pH 7.4 at 38 °C with HCl. KH1 contained (in mmol l^{-1}): NaCl 113.6, KCl 5.4, $MgCl_2$ 1.2, $NaHCO_3$ 21.0, $CaCl_2$ 1.2, Na_2HPO_4 1.5, mannitol 2.0, HEPES 20.0 and NaOH 6.0. KH2 contained (in mmol l^{-1}): NaCl 113.6, KCl 5.4, $MgCl_2$ 1.2, $NaHCO_3$ 21.0, $CaCl_2$ 1.2, Na_2HPO_4 1.5, mannitol 2.0, HEPES 7.0, gluconic acid sodium salt 6.0 and glucose 10.0. Both buffers showed an osmolarity of 296 ± 3 mOsm l^{-1}. During the experiments the buffers were continuously gassed with carbogen at 38 °C. In principle, the experimental methods for electrophysiological analysis of the recorded data were similar to those described previously (Schroeder et al. 2006; Weber et al. 2001). Briefly, short-circuit currents (I_{sc}) were recorded as a correlate of the active net transepithelial flux of electrolytes. Transepithelial electrical conductance (G_t) as calculated from Ohm's law served as a measure of tissue permeability. Transmural electrical field stimulation (EFS) of submucosal neurons in the Ussing chamber preparations was accomplished by passing electrical current between a pair of aluminum foil electrodes placed on the submucosal surface at the intersection between the two halves of the Ussing chamber. Neurogenic mediated (as shown by EFS: 16 V, 1 ms, 10 Hz, 10 s) or agonist-induced changes in I_{sc} were either expressed as maximum I_{sc} response or as the difference between the basal value before stimulation and the maximum response (ΔI_{sc}max). Forskolin and carbachol (each 10 μmol l^{-1}, serosa side) were used to pre-activate mucosa's Ca^{2+}-dependent (Chandan et al., 1991) or cAMP dependent Cl^- secretion, respectively (Kock et al., 2007; Leonhard-Marek et al., 2009).

2.4. Statistics

All data are expressed as means ± SEM. Linear regression was tested with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, U.S.A. Mean values were compared with standard *t* test for unpaired observations. A significant difference was considered at $P \leq 0.05$.

3. Results

In Exp. I the potential relationship between the basal, non-stimulated degree of electrogenic net ion transport (as indicated by baseline I_{sc}) and respective neuro-secretory response of the tissues as stimulated via EFS was tested. Under baseline conditions, sheets of distal jejunal mucosa manifested a serosa-negative I_{sc} averaging −63.7 ± 10.4 μA cm^{-2} (mean ± SEM, N = 8). Respective mean amount of EFS induced maximal I_{sc} response was 177.9 ± 28.5 μA cm^{-2}. As shown in Fig. 1, there was a significant negative correlation ($P \leq 0.001$) between the magnitude of baseline I_{sc} of mucosal tissues and the increase in I_{sc} which was inducible by EFS: the more positive the baseline I_{sc}, the smaller the pro-secretory ENS activity.

In Exp. II, baseline I_{sc} values were −49.3 ± 9.2 (N = 12) and −23.8 ± 7.7 μA cm^{-2} (N = 23; $P \leq 0.05$) in control and infected piglets, respectively (Fig. 2). Thus, in diarrhea basal I_{sc} values were significantly less negative by 52%. EFS-evoked neurogenic secretion was significantly decreased by almost

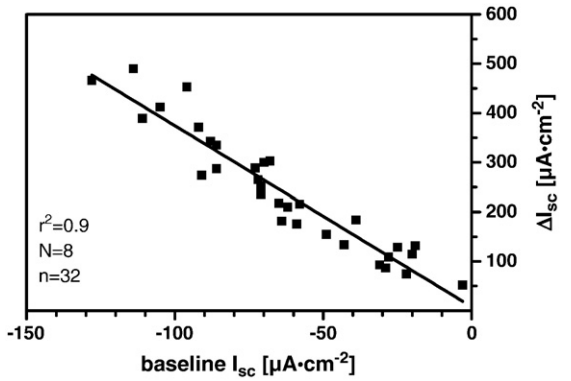

Fig. 1. The amount of the neuro-secretory I_{sc} response (ΔI_{sc}) in pig distal jejunum as induced by electrical field stimulation (EFS) as a function of non-stimulated (baseline) I_{sc}.