Effects of high levels of dietary zinc oxide on ex vivo epithelial histamine response and investigations on histamine receptor action in the proximal colon of weaned piglets


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ABSTRACT: The aim of the study was to identify the effect of high dietary zinc oxide (ZnO) levels on the histamine-induced secretory-type response and histamine metabolism in the porcine proximal colon. After weaning at d 26, 3 diets with low (LZn), normal (NZn), and high (HZn) concentrations of zinc (57, 164, or 2,425 mg/kg) were fed to a total of 120 piglets. Digesta and tissue samples were taken from the ascending colon after 7 ± 1, 14 ± 1, 21 ± 1, and 28 ± 1 d. Partially stripped tissue was mounted in Ussing chambers, and histamine was applied either to the serosal or mucosal compartments. Tissue was pretreated with or without aminoguanidine and amodiaquine to block the histamine-degrading enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HMT), respectively. Gene expression and catalytic activity of DAO and HMT in the tissue were analyzed. The numbers of mast cells were determined in tissue samples, and histamine concentration was measured in the colon digesta. Colon tissue from another 12 piglets was used for functional studies on histamine H1 and H2 receptors by using the neuronal conduction blocker tetrodotoxin (TTX) and the H1 and H2 receptor blocker chloropyramine and famotidine, respectively. After serosal histamine application to colonic tissue in Ussing chambers, the change of short-circuit current (ΔIsc) was not affected by pretreatment and was not different between Zn feeding groups. The ΔIsc after mucosal histamine application was numerically lower (P = 0.168) in HZn compared to LZn and NZn pigs. Mast cell numbers increased from 32 to 46 d of life (P < 0.05). Further studies elucidated that the serosal histamine response was partly inhibited by chloropyramine or famotidine (P < 0.01). The response to mucosal histamine tended to be decreased when chloropyramine but not famotidine was applied from either the serosal or the mucosal side (P = 0.055). Tetrodotoxin alone or in combination with chloropyramine resulted in a similar reduction in the mucosal histamine response (P < 0.01). In conclusion, the present study could not identify marked changes in colonic histamine metabolism on dietary ZnO oversupplementation. For the first time, however, H1 receptors were functionally identified in the pig colon that are localized either on neurons or on cells that activate secretion via neurons. Luminal histamine can elicit a secretory-type response via these receptors.

Key words: epithelial histamine response, histamine receptor, proximal colon, weaning pigs, zinc oxide

INTRODUCTION

Zinc oxide (ZnO) is known to reduce diarrhea in weaned piglets at so-called pharmacological dietary levels (Poulsen, 1995; Hu et al., 2014). The underlying mechanisms might be multimodal. High concentrations
of Zn decreased the release of histamine from isolated mast cells in rats in vitro (Kazimierczak and Maslinski, 1974), which is in line with the finding that high dietary Zn decreased the number of mast cells, the expression of the stem cell factor, and the histamine release in the small intestine of weaned piglets (Ou et al., 2007). Furthermore, high dietary Zn concentrations reduced the Cl− secretion to the secretagogues serotonin or theophylline in the small intestine of weaned piglets (Carlson et al., 2004). Greater systemic Zn concentrations were considered to reduce the Cl− secretion induced by serotonin (Carlson et al., 2008), carbachol, PGE2, and Escherichia coli enterotoxin in the small intestine of pigs (Gefeller et al., 2015).

However, most of the previous work focused on effects in the small intestine, and little work has been done in the colon. Possible effects in the colon of piglets might be of interest since the secretory responses to forskolin, PGE2, histamine, and carbachol were reduced in human colonic mucosa with high Zn concentrations (Medani et al., 2012).

The biogenic amine histamine has manifold functions. Besides other effects, it can act as a secretagogue, which might play a role in stress- or nutritionally induced diarrhea in young pigs.

The present study aimed to investigate the effect of three different dietary Zn levels on the histamine-induced epithelial response in the proximal colon of weaned piglets and a possible involvement of histamine-degrading enzymes. To further follow putative mechanisms, the involvement of histamine receptors H1 and H2 was studied, as there is little information available about the location and role of different histamine receptors in histamine-mediated secretory response in piglets.

**MATERIALS AND METHODS**

The study was approved by the local state office of occupational health and technical safety, Landesamt für Gesundheit und Soziales, Berlin (registration number 0347/09).

**Animals, Housing, and Diets**

A total of 120 purebred landrace piglets were weaned at 26 ± 1 d of age with a mean BW of 7.2 ± 1.2 kg. After receiving the same nonmedicated prestarter diet from 12 d of age, they were randomly allocated after weaning into 3 treatment groups balancing for gender, litter, and BW. Piglets received a wheat-, barley-, and soybean meal–based mash starter diet until 54 d of age, as described previously (Liu et al., 2014). The zinc level of the starter diet was adjusted to approximately 50 mg/kg (low dietary zinc; Lzn), 150 mg/kg (normal dietary zinc; Nzn) or 2,500 mg/kg (high dietary zinc; Hzrn) by supplementation of corn starch with analytical grade zinc oxide (Sigma Aldrich, Taufkirchen, Germany). The analysis of the diets via atomic absorption spectrometry confirmed the zinc levels (57, 164, and 2,425 mg Zn/kg, respectively). Animals were housed in stainless-steel pens (n = 2 per pen) and had ad libitum access to feed and water as described previously (Martin et al., 2013). No antibiotics were administered before or during the experiment.

**Sampling and Tissue Preparation**

The piglets were killed on 32 ± 1, 39 ± 1, 46 ± 1, and 53 ± 1 d of age (7 ± 1, 14 ± 1, 21 ± 1, and 28 ± 1 experimental days) as reported previously (Martin et al., 2013). After euthanasia, the entire intestinal tract was removed, and samples from the proximal colon were either immediately prepared for electrophysiological measurements (n = 10 per group and time point), snap frozen in liquid nitrogen for measurement of gene expression and enzyme activities (n = 8 per group and time point), or stored in zinc salt fixation solution for histological analysis (n = 6 per group and time point). The digesta of the proximal colon from Hzrn and Lzn pigs (n = 10 per group and time point) were sampled and stored at −20°C for determination of histamine concentration. The variation in the number of animals per group and time point was due to the sampling procedure and experimental setup.

Additionally, colonic tissue from 12 piglets at the age of 56 ± 7 d that received a standard weaner diet was used for functional electrophysiological analyses of histamine H1 and H2 receptors.

**Electrophysiological Measurements**

The intestinal segments of the proximal colon were immediately placed in a prewarmed and oxygenated (95% O2, 5% CO2) modified Krebs-Ringer buffer solution (pH adjusted to 7.4, containing, in moles per liter, NaCl, 0.115; KCl, 0.005; CaCl2, 0.0015; MgCl2, 0.0012; NaH2PO4, 0.0006; Na2HPO4, 0.0024; NaHCO3, 0.025; glucose, 0.01; and mannitol, 0.002). The epithelium was stripped of the serosal and muscle layers and immediately mounted in Ussing chambers with an exposed area of 1.31 cm2 and bathed in 38°C warm buffer solution. The experimental setup was as described before (Kröger et al., 2013). A microcomputer-controlled voltage/current clamp (K. M. M. S. I. U., Aachen, Germany) was used for electrical measurements. After an equilibration time of 15 to 30 min, tissues were short-circuited by clamping the voltage at 0 mV. After reaching a baseline, the diamine oxidase (DAO) blocker aminoguanidine (final concentration 100 µmol/L) and...
the histamine N-methyltransferase (HMT) blocker amodiaquine (final concentration 100 µmol/L) were added bilaterally. Additional chambers served as controls without any pretreatment. After 30 min, histamine (final concentration 100 µmol/L) was applied to the serosal compartment of the pretreated chambers and to the serosal or mucosal compartment of the untreated control chambers. The changes in short-circuit current (ΔI_{sc}) and tissue resistance (AR) were determined after histamine addition as indirect measures of electrolyte transport by subtracting the peak I_{sc} and R_{t} after 3 min from basal I_{sc} and R_{t}, respectively. Basal values (I_{sc}, R_{t}) were obtained in control chambers by calculating the mean of the last 3 min before the addition of histamine.

Tissues from the proximal colon of the 12 piglets not included in the ZnO feeding trial were used for the determination of the role of histamine receptors H1 and H2 after serosal or mucosal application of histamine using the same experimental setup as described before. Histamine was added to the serosal or mucosal side 20 min after H1 and/or H2 receptors were blocked apically or basolaterally by the H1 antagonist chloropyramine (10 µmol/L) or H2 antagonist famotidine (100 µmol/L), respectively (n = 8). Additionally, the neuronal conduction blocker tetrodotoxin (TTX; 1 µmol/L) was added to the serosal side either alone or in combination with chloropyramine (mucosal) 20 min before histamine application to the mucosal side (n = 4) to study a possible interaction of receptors with neuronal cells or their location on these cells.

RNA Extraction and Gene Expression Analysis

The extraction of RNA from colon samples by using the NucleoSpin RNAII kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was performed as described before (Kröger et al., 2013). The mRNA expression of the histamine-degrading enzymes DAO and HMT was determined by using 60S ribosomal protein L19 (RPL19) and β-2 microglobulin as housekeeping genes for data normalization (Table 1). Quantitative real-time PCR was performed using the 1-step QRT-PCR master mix kit (Brilliant II SYBR Green, Agilent Technologies, Santa Clara, CA) as described previously (Pieper et al., 2012). The obtained Ct values were normalized, and arbitrary values were calculated and used for statistical comparisons.

Determination of Enzyme Activities of DAO and HMT

The activities of DAO and HMT in colonic tissue were determined as described before (Kröger et al., 2013). Briefly, frozen tissue samples were homogenized and centrifuged for 10 min at 23,000 x g and 4°C, and the supernatants were used to determine DAO and HMT activities and protein concentrations.

Diamine oxidase activity was determined by employing a radiometric procedure with [1,4-14C]putrescine hydrochloride (0.45 mM, specific radioactivity 8.214 GBq/mol; Amersham Pharmacia Biotech, Buckinghamshire, UK) as the substrate for 30 min at 37°C as reported previously (Schwelberger et al., 1995). Background activity was measured in assays without homogenate. Histamine N-methyltransferase activity was measured by transmethylation of histamine by S-adenosyl-L-[methyl-14C]methionine (50 µM, specific radioactivity 74 GBq/mol; Amersham Pharmacia Biotech) for 30 min at 37°C (Klaus et al., 2003). Background activity was measured in assays without histamine.

The protein concentration of the homogenates was determined by the Bradford method (Bradford, 1976) using a commercially available kit (Bio-Rad Laboratories, Munich, Germany). Mean specific enzymatic activities from duplicate assays for each sample were calculated in microunits per milligram protein (µU/mg), where 1 µU converts 1 pmol substrate per minute at 37°C.

Mast Cell Counts

For histochemical staining, tissue samples of the ascending colon of 6 animals per feeding and age group were processed as described before (Rieger et al., 2013). Briefly, intestinal samples were rinsed in ice-cooled Ringer solution, cut open on the mesenteric border, trimmed, pinned with mucosal side up onto cork pieces, and immediately fixed in zinc salt fixation (González et al., 2001). Fixed tissues were dehydrated in graded series of ethanol, embedded in paraffin, cut to 5-µm thin sections, put on slides, dewaxed in xylene, rehydrated, and stained with polychromatic methylene blue modified according to Unna (Romeis, 1968).

The slides were examined under a light microscope (Axioplan, Carl Zeiss, Jena, Germany) with 400-fold magnification, and digitalized pictures were analyzed. Mast cells were counted automatically in the colonic mucosa using specific threshold settings for color, shape, and size of the metachromatic stained cells. For

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequences of primers (5’ to 3’)</th>
<th>A_{T}, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP L19</td>
<td>GCTTGCTCCCAGTGTCCTC</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>GCGTTGCGGATTTAC</td>
<td></td>
</tr>
<tr>
<td>B2M</td>
<td>CCCCAGAGTTGAGTGTAC</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>CGGAGTAGTACTGCTCCAC</td>
<td></td>
</tr>
<tr>
<td>DAO</td>
<td>GCTGAAAGCGCCCCCTTTT</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>TTGGGGGAAACTGGGGCTT</td>
<td></td>
</tr>
<tr>
<td>HMT</td>
<td>GGACGTTGTTTTCTGACCA</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>TCCTGCACTGACCTGGTTCCG</td>
<td></td>
</tr>
</tbody>
</table>

1A_{T} = annealing temperature, RPL19 = 60S ribosomal protein L19, B2M = beta-2 microglobulin, DAO = diamine oxidase, HMT = histamine N-methyltransferase.
each sample 3 slides were prepared, and an area of at least 0.5 mm$^2$ was evaluated, employing the computer-assisted image analysis program NIS-Elements AR (Nikon Instruments Inc., Melville, NY). Concentration of mast cells was measured for each sample as the number of mast cells (MC) per area (MC/mm$^2$).

### Determination of Histamine Concentration

The histamine concentration in the digesta of the proximal colon was determined only in the LZn and HZn groups as differences, if they appear, were expected to be between these two groups. The analysis was performed with ion exchange chromatography (Biochrom 20 plus Amino Acid Analyzer, Biochrom, Cambridge, UK) as described before (Pieper et al., 2012).

### Statistical Analysis

For variables, means and SEM were reported. Statistical comparisons were performed by ANOVA, except for the comparison between general $\Delta I_{sc}$ after mucosal and serosal application, which was performed using Student’s $t$ test. The level of significance was $\alpha = 0.05$. Because of the exploratory nature of the study the level of significance was not adjusted for multiple testing. All analyses were performed using IBM SPSS statistics 20 (IBM, Chicago, IL) and R version 3.0.

## RESULTS

### Baseline Values for Colonic Short-Circuit Current and Tissue Resistance

The baseline $I_{sc}$ did not differ between the feeding groups, whereas, $R_t$ was greater in HZn compared to the other groups ($P = 0.020$; Table 2).

### Histamine-Induced Changes in Short-Circuit Current and Tissue Resistance

The general $\Delta I_{sc}$ to mucosal histamine application was smaller than $\Delta I_{sc}$ after serosal histamine application (27.5 ± 2.8 and 98.7 ± 4.4 µA/cm$^2$, respectively; $P < 0.001$). No differences between feeding groups were obtained in the $\Delta I_{sc}$ after serosal histamine application. This was true for control epithelia, as well as epithelia pretreated bilaterally with aminoguanidine or amodiaquine (Table 3). Nevertheless, when histamine was added to the mucosal compartment, $\Delta I_{sc}$ was numerically lower ($P = 0.168$) in the HZn group than in the LZn and NZn groups (Table 3).

The tissue resistance decreased within 3 min after serosal application of histamine, whereas it remained nearly constant after the mucosal histamine application (Table 3). The decrease of $R_t$ after serosal histamine application was greater in the HZn group than in the NZn group, irrespective of the preincubation with AG and AD (Table 3).

### Gene Expression and Enzyme Activity of DAO and HMT in the Colon Tissue

The mRNA expression of DAO was not different between the dietary groups. The mRNA expression of HMT was lower in the HZn group than in the NZn group ($P = 0.013$), with intermediate values in the LZn
Dietary zinc and colon response to histamine

The number of mast cells did not differ between the feeding groups (180, 177, and 182 MC/mm$^2$, SEM = 4.5, for LZn, NZn, and HZn, respectively).

### Histamine Concentration in the Digesta

The concentration of histamine in the digesta of the proximal colon tended ($P = 0.092$) to be lower in piglets fed the HZn diet compared to piglets from the LZn group (23.5 and 38.5 µmol/L, SEM = 4.0, for HZn and LZn, respectively).

### Results of the Histamine Receptor Study

The colonic $I_{sc}$ response after the serosal application of histamine was reduced ($P < 0.01$) when either the H$_1$ receptor or the H$_2$ receptor was blocked serosally compared to the sole addition of histamine (Table 5).

The mucosal $I_{sc}$ response tended to be reduced by pretreatment with H$_1$ receptor antagonist chloropyramine, irrespective of whether chloropyramine was applied to the mucosal or the serosal side ($P = 0.055$; Table 5). Mucosal or serosal pretreatment with the H$_2$ receptor antagonist famotidine did not change the $\Delta I_{sc}$ induced by mucosal histamine addition.

The mucosal histamine response of the control tissue was significantly reduced ($P < 0.001$) by serosal pretreatment of tissues with the neuronal conduction blocker TTX. After combined application of TTX (serosal) and chloropyramine (mucosal or serosal), $\Delta I_{sc}$ after histamine application was no longer different from zero (Table 6).

### DISCUSSION

Previous work concerning the effect of zinc on the secretory response of the intestinal epithelium was mainly based on enterotoxins or secretagogues that are mediators of the diarrheal response to enterotoxins in the small intestine, e.g., serotonin (Carlson et al., 2004, 2008; Gefeller et al., 2015). Similarly, Zn effects on toxin-induced ion secretion were studied in human Caco-2 cells (Canani et al., 2005). Because recent data suggest an interaction of zinc with the response of the human colonic mucosa to histamine (Medani et al., 2012), we aimed to study this interaction in more detail in the proximal colon in pigs. Histamine is well known to induce chloride

<table>
<thead>
<tr>
<th>Item</th>
<th>LZn</th>
<th>NZn</th>
<th>HZn</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
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<tr>
<td>DAO expression</td>
<td>1.3</td>
<td>1.4</td>
<td>1.8</td>
<td>0.1</td>
<td>0.274</td>
</tr>
<tr>
<td>$n$</td>
<td>30</td>
<td>29</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMT expression</td>
<td>1.0</td>
<td>1.3</td>
<td>0.9</td>
<td>0.1</td>
<td>0.013</td>
</tr>
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<td>$n$</td>
<td>26</td>
<td>22</td>
<td>27</td>
<td></td>
<td></td>
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<tr>
<td>DAO activity, µU/mg protein</td>
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<td>32.9</td>
<td>44.5</td>
<td>3.1</td>
<td>0.111</td>
</tr>
<tr>
<td>$n$</td>
<td>31</td>
<td>32</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMT activity, µU/mg protein</td>
<td>409</td>
<td>404</td>
<td>397</td>
<td>9.4</td>
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<td>32</td>
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<td></td>
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</tbody>
</table>

$^{a,b}$Different superscripts within a row indicate differences between feeding groups ($P < 0.05$).

$^1$LZn = low zinc level, 57 mg zinc/kg; NZn = normal zinc level, 164 mg zinc/kg; HZn = high zinc level, 2,425 mg zinc/kg.

$^2$Arbitrary values.

<table>
<thead>
<tr>
<th>Item</th>
<th>Histamine, serosal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloropyramine, serosal</td>
<td>Famotidine, serosal</td>
</tr>
<tr>
<td>$\Delta I_{sc}$, µA/cm$^2$</td>
<td>72.1</td>
</tr>
</tbody>
</table>

$^{a,b}$Different superscripts indicate differences ($P < 0.05$).

### Table 5. Colonic tissue short-circuit current response ($\Delta I_{sc}$) 3 min after the serosal application of histamine (control; 100 µmol/L) with or without serosal pretreatment with chloropyramine (10 µmol/L) or famotidine (100 µmol/L) and 3 min after the mucosal application of histamine (100 µmol/L) without or with mucosal or serosal pretreatment with chloropyramine (10 µmol/L; $n = 8$)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TTX, serosal</th>
<th>Chloropyramine, mucosal</th>
<th>TTX, serosal and Chloropyramine, mucosal</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta I_{sc}$, µA/cm$^2$</td>
<td>7.0</td>
<td>2.3</td>
<td>-0.4</td>
<td>0.1</td>
<td>0.9</td>
<td>&lt;0.001</td>
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</tbody>
</table>

$^{a,b}$Different superscripts indicate differences ($P < 0.05$).

$^1$TTX = tetrodotoxin.
secretion into the gut lumen, which can be visualized in vitro by an increase of $I_{sc}$ in Ussing-chambered intestinal epithelia (Traynor et al., 1993; Ahrens et al., 2003). Histamine is very special among many secretagogues because it is of interest with regard to diarrhea in weanling piglets as it not only can be released from mast cells, e.g., as an immune reaction, but can also be produced in the colonic lumen by bacteria via the decarboxylation of the amino acid histidine. Diets with high levels of low digestible protein have been identified as 1 factor that increases the histamine concentration in the colonic digesta of piglets (Schneider et al., 1989; Pieper et al., 2012). Although diets with the same protein composition were fed to all groups in the current study, a tendency of a lower histamine concentration was observed in the colonic digesta of piglets from the HZn group compared to piglets from the NZn. Such numerically lower colonic histamine concentrations in the high-Zn group may be due to altered microbiota composition, which has been shown to respond to ZnO addition to the diet (Vahjen et al., 2011), including for the animals from this study (Starke et al., 2014). Also, the number of mast cells did not differ between feeding groups, indicating that dietary zinc level did not affect mast cells in the porcine proximal colon.

The question of whether altered luminal histamine bioavailability also induces long-term (i.e., chronic) changes in colonic epithelial function or whether such changes in colonic epithelia function are attributable to the luminal presence of Zn per se was beyond of the scope of this study. However, the chronic impact of Zn supplementation on colonic mucosa function finally proved to be rather minor. The term “chronic effects of Zn” is used to underline that the epithelia was not exposed to Zn during the in vitro investigations. All Zn effects measured in vitro are thus attributable to Zn preexposure in vivo and not to the actual presence of Zn. Interestingly, 1 of the minor impacts of high zinc supplementation was a lower increase of $I_{sc}$ when colonic tissues from the HZn group were exposed to histamine on the mucosal side. A secretory-type response to mucosally applied histamine is rather unusual in the literature and was first reported in the porcine proximal colon (Aschenbach et al., 2007). In that study, however, the $I_{sc}$ responses after mucosal histamine application were much smaller than that in the present study and appeared only after transportation stress.

Because of this rather unexpected result, we performed further functional studies. Serosal pretreatment with the H$_1$ and H$_2$ receptor antagonists chloropyramine and famotidine equally reduced the $I_{sc}$ response to serosal histamine. This could indicate that both H$_1$ and H$_2$ receptors are involved in the histamine response in the porcine proximal colon. Even so the involvement of both receptors is not unusual when considering results from other colonic tissues (Rangachari and Prior, 1994; Keely et al., 1995), including the porcine distal colon (Traynor et al., 1993), it is in sharp contrast to previous results in the porcine proximal colon in which H$_2$ receptors dominated the histamine response (Ahrens et al., 2003). The obvious difference between the present investigation and the previous study was the preparation of the tissue. In the present study, partially stripped colonic epithelia were used, whereas slide-stripped epithelial preparations were used in the previous study (Ahrens et al., 2003). The different methods of tissue preparation promoted a suspicion that the H$_1$-mediated component of histamine action may be dependent on the tissue immediately underlying the epithelium.

The H$_1$ receptors in the human intestinal tract are known to be located on ganglion cells of the myenteric plexus, on epithelial cells, on intestinal fibroblasts, and on the muscular layers (Sander et al., 2006). In the canine proximal colon, H$_1$ receptors are primarily responsible for neuronal effects and are presumably located in the submucosal plexus (Rangachari and Prior, 1994). As such, the H$_1$-mediated component of histamine action declines in an aganglionic epithelium (Rangachari and Prior, 1994). The same appears to be true for the porcine proximal colon of piglets and may explain the differences in the mucosal histamine response between the present and previous studies (Aschenbach et al., 2007). In the present study, the response to mucosally added histamine was reduced or inhibited after the pretreatment with TTX, chloropyramine, and a combination of both, suggesting an involvement of H$_1$ receptors located on neuronal cells or on cells that evoke secretion via neurons.

Interestingly, the H$_1$-mediated $I_{sc}$ response after mucosal histamine application was blocked by not only mucosal but also serosal pretreatment with chloropyramine. Although we cannot rule out that H$_1$ receptors are also located on the luminal side of the colonic epithelium, the parallel effectiveness of TTX to reduce the response to mucosal histamine application is a strong argument in favor of the H$_1$ receptors being located on nerves beneath the epithelium. As most of the classical H$_1$ receptor antagonists are known to cross the blood-brain barrier (Hill et al., 1997), it can be hypothesized that chloropyramine partially crossed the intestinal barrier when applied from the mucosal side and inhibited H$_1$ receptors on neurons underneath the epithelium or their projections into the paracellular space.

It is known that the tightness of the paracellular space can be increased by pharmacological dosages of zinc as a result of enhancing the expression of tight junction proteins in the jejunal (Hu et al., 2013a) or ileal (Zhang and Guo, 2009) mucosa. This results in an increased transepithelial electrical resistance (Hu et al., 2013b). Such increased $R_t$ was also observed in the HZn
group compared to the LZn and NZn groups in the present study, which might indicate a tighter paracellular barrier. In contrast, a tighter paracellular barrier could well explain the reduced epithelial response to mucosal histamine when assuming that histamine has to cross the tight junctions to reach the H₁ receptors of neurons on the other side of the epithelium. The latter concept would also accommodate the finding that prefeeding high concentrations of Zn affected only the $J_{sc}$ response after mucosal but not serosal histamine application, the latter also being dependent on H₁ receptors.

As pointed out earlier, the present study investigated only the chronic effects of Zn exposure but not its acute effects. Previous studies have shown that the actual physical presence of zinc on the serosal side can inhibit the cyclic adenosine monophosphate (cAMP)-stimulated chloride secretion in the rat ileum by blocking serosal K⁺ channels (Hoque et al., 2005). A similar effect can be expected for the proximal colon of piglets because the present and previous studies have shown a strong involvement of H₂ receptors in the secretory-type response of this epithelium (Ahrens et al., 2003); H₂ receptors activate the apical chloride channel cystic fibrosis transmembrane conductance regulator predominantly via the cAMP pathway (Hill et al., 1997; Barrett and Keely, 2000). Exploring the acute effects of Zn in detail was beyond the scope of the present study because these effects would not be specific to histamine but applicable to any secretagogue. In a parallel study on jejunal epithelia from the same piglets, it was shown that the addition of 24 µmol/L ZnSO₄ to the serosal buffer solution in vitro decreased the secretory-type responses to serosal addition of PGE₂ and carbachol and the mucosal application of E. coli heat-stable enterotoxin independent of the level of ZnO prefeeding in vivo (Gefeller et al., 2015). The latter supports the general concept that zinc needs to be absorbed to increase zinc concentration in the basolateral compartment of the intestine to reduce the response to secretagogues (Carlson et al., 2008; Medani et al., 2012), including histamine. In this study these conditions can be assumed, at least for the HZn group, because it was already reported that the serum concentration of zinc was greater ($P < 0.001$) when the piglets used in this study were fed the high-zinc diet compared to the low- or medium-zinc diets, independent of the age (Martin et al., 2013).

Previous studies have suggested that altered epithelial responses to histamine may result from altered activity of histamine-degrading enzymes (Aschenbach et al., 2007; Kröger et al., 2013). The main enzymes involved in histamine degradation in the porcine colon are HMT and DAO (Aschenbach et al., 2006). Therefore, the involvement of HMT and DAO in the histamine effect was tested, along with the gene expression and enzyme activity of both enzymes in the colonic tissue. No differences were observed in the change of $J_{sc}$ between the feeding groups after serosal application of histamine with or without blocking the histamine-degrading enzymes DAO and HMT. Therefore, the results of this study do not indicate that the secretory-type response of the colonic epithelium was modulated by changes in enzyme activity of DAO or HMT due to differing zinc concentrations in the diet. This was confirmed by demonstrating that gene expression and enzyme activity of DAO, as well as enzyme activity of HMT, were not different between the feeding groups. There appeared to be some minor downregulation of HMT mRNA in the HZn group that, however, did not translate into decreased HMT activity. Considering that pharmacological dosages of zinc decreased the activity of DAO in the serum but had no effect on DAO activity in the jejunal mucosa of weanling piglets (Hu et al., 2014), it can be suggested that an altered activity of histamine-degrading enzymes is not part of the gastrointestinal response to high-dose Zn supplementation in piglets.

In conclusion, the present study did not identify any significant changes in colonic histamine metabolism and mast cell number on dietary ZnO oversupplementation. However, the data indicate that diets high in zinc appear to reduce the ex vivo secretory-type response of colonic epithelia from weaned piglets after histamine application from the luminal side. The latter response was sensitive to tetrodotoxin and chloropyramine and can be attributed to H₁ receptors localized either on neurons or on cells that activate secretion via neurons. Effects of serosal histamine application are mediated by both H₁ and H₂ receptors and do not appear to be chronically altered by prefeeding a high-zinc diet.

**LITERATURE CITED**


