Serosal zinc attenuate serotonin and vasoactive intestinal peptide induced secretion in piglet small intestinal epithelium in vitro

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Abstract

This study addressed the mechanisms by which dietary zinc affects diarrhoea and aimed to study possible interactions between zinc status and the presence of zinc in vitro on secretagogue-induced secretion from piglet intestinal epithelium in Ussing chambers. In addition, it was studied from which side of the epithelium zinc would perform an effect and if copper caused similar effects. Twenty-four piglets (28 days of age) were weaned and fed diets containing 100 or 2500 mg zinc/kg (as ZnO) for 5 or 6 days (12 piglets per group). Intestinal epithelium underwent the following 5 treatments: zinc at the mucosal side (MZn), zinc at the serosal side (SZn), zinc at both sides (MSZn), copper at both sides (MSCu) or water at both sides (control). Provoked secretion in terms of short circuit responses to serotonin (5-HT) and vasoactive intestinal peptide (VIP) were measured. Zinc at the serosal or both sides of the epithelium reduced the 5-HT induced secretion (P<0.001); however, due to interactions (P=0.05) the effect of zinc in vitro was only present in the ZnO100 group. The secretion caused by VIP was not affected by the diet (P=0.33), but zinc at the serosal side or both sides reduced the response to VIP (P<0.001). Copper reduced the 5-HT and VIP induced secretion to a larger extent than zinc. However, copper also disturbed intestinal barrier function as demonstrated by increased transepithelial conductance and increased short circuit current, which was unaffected by zinc. In conclusion, zinc at the serosal side or both sides of the epithelium has to be increased to reduce secretagogue-induced chloride secretion and thereby diarrhoea.

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Keywords: Copper; Zinc; Diarrhoea; Piglet; Ussing chamber; Weaning; Metallothionein; Alkaline phosphatase

1. Introduction

In zinc deficiency, the organism is more sensitive to toxin producing bacteria that stimulate chloride secretion and cause diarrhoea (Wapnir et al., 2000) and it is well known that dietary zinc treatment has a preventive effect on diarrhoea in newly weaned piglets (Poulsen, 1995). Pigs are often used as animal models to study human intestinal physiology (Grøndahl et al., 2002) and understanding of how supplementation with zinc alleviates diarrhoea in weanling pigs may have valuable implications for both animal and human nutrition and medicine. Dietary zinc (Zn) has a preventive impact on diarrhoea in newly weaned piglets (Poulsen, 1995), but the mechanisms behind are still not completely understood. When enterotoxins are secreted from pathogenic bacteria in the intestinal lumen, the neurotransmitter 5-HT is released from cells located in the intestinal epithelium and a range of receptors at the epithelial cells are activated resulting in chloride (Cl−) secretion (Skadhauge et al., 1997). In addition, 5-HT promotes the release of other neurotransmitters (e.g. vasoactive intestinal peptide, VIP) from enteric nerve endings, which elevates intracellular messengers (e.g. cAMP) followed by Cl− secretion in vitro. These in vitro studies indicate that in vivo there will be no positive acute effect of increasing luminal Zn concentration on secretagogue-induced chloride secretion and that zinc status at the serosal side of the epithelium has to be increased to reduce secretagogue-induced chloride secretion and thereby diarrhoea.
zinc directly to the bathing media around the intestinal epithelium in Ussing chambers (Carlson et al., 2006). In vitro studies with rat intestinal epithelium performed by Hoque et al. (2005) showed that zinc at the serosal side reduced the cAMP-dependent chloride secretion caused by forskolin. However, a study on caco-2 cells showed that both mucosal and serosal zinc affected the chloride secretion (Canani et al., 2005). The results from our previous study (Carlson et al., 2006) suggested that the inhibitory mechanism of zinc ions takes place at receptors situated at the basolateral membrane of the epithelial cells. However, the latter study left it open for discussion whether the anti-secretory effect of zinc is unique for zinc ions or if the presence of other divalent cat ions would cause the same effects.

Our previous findings were further explored in the present study by examining if an attenuating effect of zinc on secretagogue provoked Cl− secretion from piglet small intestinal epithelium measured in vitro was affected by the level of dietary zinc consumed before slaughter. In addition, the aim was to determine from which side of the epithelium zinc performs its anti-secretory effect and to study if this effect is unique for zinc or if another divalent cat ion, like copper, can attain the same effect. The secretagogues used to activate Cl− secretion from piglet small intestinal epithelium was 5-HT and VIP.

2. Materials and methods

2.1. Animals and Diets

The protocol used in the present experiment complied with the Danish Ministry of Justice concerning animal experimentation and care of experimental animals.

The experiment included 24 Landrace/Yorkshire/Duroc cross-bred piglets (Sus scrofa) (6 litters of 4 piglets) (2 gilts and 2 castrates per litter) with an average mass of 8.7 (±1.3) kg at weaning. They were allowed access to feed and water. At weaning the piglets were allocated randomly, within litter on a basis of sex and body weight to one of two dietary treatments (Table 1). The basal diet was analyzed for dry matter and zinc content of the two experimental diets (ZnO100 and ZnO2500).

2.2. Sample collection

Immediately after the piglets were euthanized, a midline abdominal incision was made and 30 cm of the small intestine (located 5 m proximal to the ileocecal junction) was removed and placed in an oxygenated and phosphate-buffered Ringer solution at room temperature. The Ringer solution contained in mmol/L: 25 NaHCO3, 120 NaCl, 1.0 MgSO4, 6.3 KCl, 2.0 CaCl, 0.32 phosphate buffer (pH 7.4) and 16 glucose. This site of the small intestine was used for electrophysiological measurements in vitro. For metallothionein (MT), zinc and copper measurements another 100 cm of the mid-jejunum (located proximal to the first site) was cut open and washed with cooled saline. The mucosa of this intestinal segment was scraped from the underlying muscular layers prior to MT, zinc and copper analysis. In addition, the liver from each piglet was collected for zinc, copper and MT analyses. Intestinal mucosa and liver samples were stored at −20 °C.

2.3. Measurement of electrophysiological parameters

Within 15 min after the piglets were euthanized, the epithelium was stripped of the muscle layers. The stripped epithelial sheets were mounted in Ussing chambers (WPI, Sarasota, FL, USA) in 8 replicates and 10 mL Ringer solution was used as bathing medium on each side. The Ussing chamber procedure was used as described by Carlson et al. (2006). The bathing media were replaced 10 min after mounting the tissue. After replacing the bathing media, the tissues equilibrated for 15 min, before basal short circuit current (Isc) and potential difference (PD) were recorded. Conductance (G) was calculated from Isc and PD by Ohms law. Five min thereafter, 200 μL of a ZnSO4 solution was added at the mucosal side to 2 chambers (MSzn), at the serosal side to 2 chambers (Szn) or bilaterally to 2 chambers (MSzn) (chamber zinc concentration 0.023 mmol/L).

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Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Dietary ingredients (g/kg) and analyzed zinc and copper (mg/kg DM) of the basal diet and analyzed dry matter and zinc content of the two experimental diets (ZnO100 and ZnO2500)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Barley</td>
</tr>
<tr>
<td>ZnO100</td>
<td>312.7</td>
</tr>
<tr>
<td>ZnO2500</td>
<td>91.5</td>
</tr>
</tbody>
</table>

* Zinc-free vitamin–mineral mixture provided the following quantities of vitamins and minerals per kg of complete diet: 4400 IU of vitamin A, 1000 IU of vitamin D3, 60 mg of α-tocopherol, 2.2 mg of menaphthone, 2.2 mg of thiamin, 4 mg of riboflavin, 11 mg of p-panthotenic acid, 22 mg of niacin, 0.06 mg of biotin, 0.022 mg of cyanocobalamin, 3.3 mg of pyridoxin, 50 mg Fe as FeSO4, 20 mg of Cu as CuSO4·5H2O, 27.7 Mn as MnO, 0.21 as Kl, 0.3 Se as Na2SeO3.
Furthermore, in 1 chamber 200 μL of a CuSO₄ solution was supplemented bilaterally (MSCu) (chamber copper concentration 0.023 mmol/L). The MSZn treatment was considered as a positive control (a divalent cation). In the remaining chamber 200 μL of water was added (control). Due to a low solubility of ZnO it was chosen to use a highly soluble ZnSO₄ in the in vitro studies.

To determine changes in Isc and G due to zinc and copper supplementation, basal Isc and PD were recorded again fifteen min subsequent to zinc and copper supplementation. Five minutes thereafter, 0.1 mmol/L of 5-HT (Sigma, Copenhagen, Denmark) was added at the serosal side of all chambers followed twenty min later by 0.1 μmol/L of VIP (Calbiochem, Albertslund, Denmark), also at the serosal side.

2.4. Chemical analyses

Samples of whole blood were centrifuged at 12,620 g for 3 min (Sigma 201 M, Osterode am Harz, Germany) and haematocrit was measured. To determine mineral concentrations, the diets, mucosa and liver samples were homogenized and dried at 100 °C followed by ashing at 450 °C. The ash was digested in a 21.7% nitric acid solution. The plasma samples were wet ashed in nitric acid (14.4 M) and perchloric acid (12 M) at 200 °C. The concentrations of zinc and copper were determined by atomic absorption spectrophotometry (Unicam SP9, Phillips, Cambridge, UK). Alkaline phosphatase (AP) activity in plasma was measured by kinetic photometry (Hitachi 912, Roche Diagnostics, Mannheim, Germany) (Tietz et al., 1983).

The MT protein concentration in intestinal mucosa and liver was determined by a modification of the silver saturation assay (Scheuhammer and Cherian, 1991). Red blood cell (RBC) haemolysate was prepared from fresh porcine blood (Onosaka and Cherian, 1982). Tissue (0.45 g) was homogenized (Ultra Turrax, IKA-Werke, Staufen, Germany) in 250 mmol/L sucrose buffer (1:4) and centrifuged at 18,000 g for 20 min. The supernatant (50–400 μL) was dispensed into glass test tubes and 0.5 mol/L glycine buffer was added to make 0.8 mL. Subsequently, 0.5 mL of a 20 μg/g Ag⁺ solution was added and incubated at room temperature for 5 min, then 100 μL RBC haemolysate was added and the samples were mixed. After 1.5 min at 100 °C in a glycercine bath, the samples were centrifuged at 1200 g (room temperature). Another 100 μL RBC haemolysate was added and the heating/centrifugation step was repeated. Finally, the samples were centrifuged at 15,000 g for 5 min at room temperature. The silver concentration of the final supernatant was measured by atomic absorption spectrophotometry (AAS) (Unicam SP9, Phillips, Cambridge, UK). An AAS standard curve for silver was generated using known amounts of silver in glycine buffer.

2.5. Statistical analysis

Statistical analysis was carried out by the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). The effect of dietary zinc on growth performance, zinc status, and basal transport parameters were analyzed by the following model:

\[ Y_{dle} = \mu + \alpha_d + U_1 + \epsilon_{dle} \]

Using chamber data were analysed by the following model:

\[ Y_{dle} = \mu + \alpha_d + \beta_z + X_{lp} + U_p + \epsilon_{dle} \]

For each variable, the validity of the analysis was controlled (Scheuhammer and Cherian, 1991). The basal Isc and G were determined by a modification of the silver saturation assay (Scheuhammer and Cherian, 1991). Red blood cell (RBC) haemolysate was measured by a modification of the silver saturation assay (Scheuhammer and Cherian, 1991).

Table 2

Effect of dietary zinc (100 or 2,500 mg zinc/kg, as ZnO) on feed intake, growth rate, haematocrit and zinc and copper status indicators in plasma, mucosa, and liver from piglets 5 to 6 d after weaning.

<table>
<thead>
<tr>
<th>Diet</th>
<th>ZnO₁₀₀</th>
<th>ZnO₂₅₀₀</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mass, kg</td>
<td>8.8</td>
<td>8.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Growth rate, g/d</td>
<td>–60</td>
<td>–5</td>
<td>36</td>
<td>0.31</td>
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<tr>
<td>Feed intake, g/d</td>
<td>210</td>
<td>133</td>
<td>38</td>
<td>0.07</td>
</tr>
<tr>
<td>Haematocrit, %</td>
<td>42</td>
<td>41</td>
<td>1.4</td>
<td>0.7</td>
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</table>

Plasma

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<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Zinc, mg/L</td>
<td>0.3</td>
<td>1.0</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Copper, mg/L</td>
<td>1.6</td>
<td>1.7</td>
<td>0.1</td>
<td>0.34</td>
</tr>
<tr>
<td>AP⁺, U/L</td>
<td>118</td>
<td>138</td>
<td>7</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Mucosa

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</thead>
<tbody>
<tr>
<td>Zinc, μg/g DM</td>
<td>100</td>
<td>124</td>
<td>7</td>
<td>0.03</td>
</tr>
<tr>
<td>Copper, μg/g DM</td>
<td>9.8</td>
<td>9.7</td>
<td>0.4</td>
<td>0.85</td>
</tr>
<tr>
<td>MT⁺⁺, μg Ag/g</td>
<td>2.8</td>
<td>3.3</td>
<td>0.3</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Liver

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<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Zinc, μg/g DM</td>
<td>300</td>
<td>453</td>
<td>24</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Copper, μg/g DM</td>
<td>103</td>
<td>94</td>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>MT, μg Ag/g</td>
<td>184</td>
<td>325</td>
<td>32</td>
<td>≤0.001</td>
</tr>
</tbody>
</table>

Values are least square means (n=12 piglets) and P-value represent the effects of dietary zinc.

a AP⁺⁺= alkaline phosphatase

b MT⁺⁺= metallothionein
significant at $P \leq 0.05$. The pair-wise comparison procedure in SAS was used to separate the least square means.

3. Results

The zinc concentration in the ZnO$_{100}$ diet was at the expected level with contributions from feed ingredients and from the ZnO source, whereas the ZnO$_{2500}$ diet was slightly below the expected level (Table 1). During the 5 to 6 d experimental period none of the piglets showed signs of post-weaning diarrhoea. Piglet performance in terms of growth rate did not differ between the two dietary treatments ($P=0.31$), but there seemed to be a tendency for reduced feed intake in the ZnO$_{2500}$ group ($P=0.07$) (Table 2). When comparing the two experimental groups, the zinc concentration in plasma, intestinal mucosa and liver increased with increased dietary zinc concentrations ($P=0.02, P=0.03$ and $P \leq 0.0001$, respectively). The AP activity in plasma tended to increase due to the high ZnO concentration ($P=0.07$). The MT protein was almost twice as high in liver from the ZnO$_{2500}$ group compared to the ZnO$_{100}$ group ($P \leq 0.001$), whereas the MT concentration in the intestinal mucosa was not affected by the dietary treatments ($P=0.25$).

The basal $I_{sc}$ and $G$ measured 5 min before zinc or copper was added to the bathing media were not affected by the dietary treatments ($P=0.12$ and $P=0.95$, respectively) (Table 3).

<table>
<thead>
<tr>
<th>Diet</th>
<th>$P$-value</th>
<th>ZnO$_{100}$</th>
<th>ZnO$_{2500}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isc (μA/cm$^2$)</td>
<td>159 (128–198)</td>
<td>179 (144–222)</td>
<td>0.12</td>
</tr>
<tr>
<td>$G$ (mS/cm$^2$)</td>
<td>33 (±1.2)</td>
<td>33 (±1.2)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Values are LS-means with 95% confidence intervals or SEM and $n=96$ tissues (from 12 piglets) for each diet.

Fig. 1. The change in short circuit current ($\Delta I_{sc}$) (a) and the change in conductance ($\Delta G$) (b) after zinc or copper supplementation ($\Delta$ is the change from 5 min before to 15 min after zinc or copper supplementation). $\Delta I_{sc}$ was not affected ($P=0.16$) by dietary treatment but by Ussing chamber treatment ($P \leq 0.0001$) and $\Delta G$ was not affected by dietary treatment ($P=0.81$) but by Ussing chamber treatment ($P \leq 0.0001$). There were no interactions between dietary and Ussing chamber treatments. Dietary treatments were 100 mg zinc/kg (ZnO$_{100}$) or 2500 mg zinc/kg (ZnO$_{2500}$) and Ussing chamber treatments were zinc or copper supplementation (0.023 mmol/L) at the mucosal (M), serosal (S) or both (MS) sides of the intestinal epithelium Values are LS-means and error lines represent SEM. For MZn, S$_{Zn}$ and MS$_{Zn}$, n=48 tissues from 24 piglets and for control and MS$_{Cu}$, n=24 tissues from 24 piglets. Values with different letters differ ($P \leq 0.05$).
Fifteen min after in vitro treatments (addition of water (control), Zn or Cu) basal Isc and G had increased (Fig. 1). However, copper increased Isc and G significantly more than both zinc and control treatments. The level of amplification of Isc and G after water, zinc or copper supplementation was not affected by the previous dietary treatments \((P = 0.16\) and \(P = 0.81\). respectively).

When 5-HT was added to the chambers with zinc supplemented at the serosal or both sides of the epithelium the induced secretion was attenuated \((P < 0.001)\); however, this was only significant when piglets had been fed the ZnO100 diet (Fig. 2). When copper was added to the bathing media, the secretory response to 5-HT was further decreased and this attenuating effect was happening independent on previous dietary treatment. The secretion caused by VIP was not affected by dietary treatments \((P = 0.33)\) (Fig. 3); however, when zinc was supplemented at the serosal side or both sides of the epithelium the secretory response was reduced compared to adding zinc only at the mucosal side. However, the secretory response induced by VIP was reduced the most when copper

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**Fig. 2.** The effect of dietary treatment \((P = 0.38)\) and Ussing chamber treatment \((P < 0.001)\) and their interactions \((P = 0.05)\) on changes in short circuit current induced by 0.1 mmol/L of 5-HT. Dietary treatments were 100 mg zinc/kg (ZnO100) or 2500 mg zinc/kg (ZnO2500) and Ussing chamber treatments were zinc or copper supplementation \(0.023 \text{ mmol/L}\) at the mucosal (M), serosal (S) or both (MS) sides of the intestinal epithelium. Values are LS-means and error lines represent SEM. For MZn, SZn and MSZn \(n = 48\) tissues from 24 piglets and for control and MSCu \(n = 24\) tissues from 24 piglets. Values with different letters differ \((P \leq 0.05)\).

**Fig. 3.** The effect of dietary treatment \((P = 0.33)\) and Ussing chamber treatment \((P < 0.001)\) and their interactions \((P = 0.55)\) on changes in short circuit current induced by 0.1 μmol/L of VIP. Dietary treatments were 100 mg zinc/kg (ZnO100) or 2500 mg zinc/kg (ZnO2500) and Ussing chamber treatments were zinc or copper supplementation \(0.023 \text{ mmol/L}\) at the mucosal (M), serosal (S) or both (MS) sides of the intestinal epithelium. Values are LS-means and error lines represent SEM. For MZn, SZn and MSZn \(n = 48\) tissues from 24 piglets and for control and MSCu \(n = 24\) tissues from 24 piglets. Values with different letters differ \((P \leq 0.05)\).
was supplemented to the bathing media. These attenuating effects of zinc and copper on the VIP response were independent of the dietary treatments as there were no interactions between zinc and copper in the bathing media and the dietary treatments.

4. Discussion

In the present study the dietary zinc source was ZnO, as high zinc concentrations from ZnO is known to reduce post-weaning diarrhoea (Carlson et al., 2004). However, ZnO has a very low solubility in water compared to e.g. ZnSO₄. Consequently, as complete solubility is necessary for successful Ussing chamber experiments, ZnSO₄ was used as the zinc source in the presented in vitro studies.

One of the biggest challenges when performing in vitro studies is to imitate the in vivo situation e.g. using relevant physiological concentrations. In the present study, a zinc and copper concentration of 0.023 mM was used, as it is on level with zinc concentrations found in plasma from piglets fed above 2000 mg zinc/kg diet (Carlson et al., 1999; Carlson et al., 2007) and it is the plasma zinc concentration being associated with the positive effect of zinc on weight gain in pigs (Hahn and Baker, 1993). Furthermore, in a doses–response study this zinc concentration attenuated secretagogue-induced secretion to the greatest extent (Carlson et al., 2006). The average zinc concentration in plasma from the present piglets was 0.0046 mM and 0.015 mM in the ZnO₁₀₀ and ZnO₂₅₀₀ group, respectively and the copper concentration in plasma was 0.027 mM, irrespective of treatment. However, as the bathing media used in the Ussing chambers lacks the plasma proteins (albumin) and amino acids, the concentration of zinc and copper in ionic form in the in vitro studies may have been high compared with corresponding concentrations in plasma.

The basal transport (Isc and G) measured before zinc, copper or secretagogues were added to the bathing media were not affected by the dietary zinc concentrations, which supports previous studies (Carlson et al., 2004; Feng et al., 2006). However, the basal Isc and G were increased 15 min after supplementation of 0.023 mM of copper, whereas the increase in Isc and G in epithelium from the zinc treated epithelium did not differ from the controls. These results show that adding 0.023 mM of copper ions disturbed the basic Isc and G in the epithelial tissues. Whether the increase in Isc was due to increased Na⁺ absorption, increased Cl⁻ secretion or maybe copper transport is difficult to interpret from this study. The increase in G may indicate that the copper treatment resulted in a leakier epithelium. In an Ussing chamber study with Caco-2 cells physiologically high zinc concentrations (0.2 mM) increased Isc (Canani et al., 2005). Consequently, the present study may indicate that 0.023 mM of copper largely in ionic form is too high or even toxic to the intestinal epithelium. However, whether the copper had toxicological effects on the epithelium was not studied further in the present study. Tissues incubated with copper responded less to 5-HT and VIP compared with other incubations. The reducing effect of copper on induced secretion compared with zinc seems to be in contrast with observations in vivo, where dietary zinc appears to have a much greater influence on diarrhoea compared with dietary copper (Carlson et al., 2004). Consequently, the observed effect of copper on 5-HT and VIP responses in vitro may be an artefact caused by toxic effects of copper on the epithelium. Another challenging result in respect of interpretation is the increase in Isc 15 min after 200 μl water was added to the control chambers in the ZnO₂₅₀₀ group compared with the ZnO₁₀₀ group. However, the control tissue from the ZnO₂₅₀₀ group did not differ from the other in vitro treatments in this group, thus these findings may indicate a generally higher increase in active ion transport with time in tissue from the ZnO₂₅₀₀ group compared with the ZnO₁₀₀ group. However, the differences between dietary groups were not statistical significant. In a similar study Isc also increased numerically more with time in epithelial tissue from a ZnO₂₅₀₀ group compared with a ZnO₁₀₀ group (Feng et al., 2006), however, also in this study the difference was not statistical significant.

This study offers new insight into the role of zinc in prevention of post-weaning diarrhoea in piglets and extends our previous findings concerning the actions of zinc on responses to different secretagogues in piglet small intestinal epithelium.

From Figs. 2 and 3 it is clear that zinc exerts its attenuating effect on secretion from the basolateral side of the epithelium, which was also shown in rat tissue (Hoque et al., 2005). This may indicate that dietary zinc needs to be absorbed and even circulated in the blood before it is able to reduce secretion. However, the exact mode of action for zinc at the intestinal level is still not clear. In the following, four possible mechanisms behind the attenuating effect of zinc observed in vitro are proposed and discussed. As zinc and copper are recognized as neurotransmitters in the central nervous system (Frederickson and Bush, 2001), it is obvious to suggest that the enteric nervous system (ENS) is involved in the effect of zinc and copper on induced secretion. However, another study indicated that the attenuating effect of zinc on cAMP dependent (induced by forskolin) secretion from small intestinal epithelium was not affected by blockage of the ENS (Feng et al., 2006). In human embryonic kidney (HEK 293) cell lines, 5-HT₃ receptors were affected by zinc resulting in increased electrophysiological responses to 5-HT at a zinc concentration of 0.003 mmol/L and reduced responses at a zinc concentration of 0.3 mmol/L (Gill et al., 1995). Furthermore, in the same type of cells the effect of zinc on the response to 5-HT was found to be modulated by other divalent cat ions (Ca²⁺ and Mg²⁺), suggesting that there is more than one divalent cation binding site at the 5-HT receptor molecule (Hubbard and Lummis, 2000). Consequently, the attenuating mechanism of zinc and copper on secretion in the present study may be due to binding at basolateral 5-HT receptors. However, the bathing media used in the present study did contain Ca²⁺ (2 mM) and Mg²⁺ (1 mM), and consequently if divalent cation binding sites at the basolateral receptors are responsible for the attenuating effect of zinc and copper, some of these binding sites must be specific or have higher affinity for zinc and copper compared with Ca and Mg. In this light, the more pronounced effect of copper compared to zinc may indicate that there are different affinities for zinc and copper.
Hoque et al. (2005) suggested that zinc selectively inhibited cAMP-induced chloride secretion by blocking basolateral K
channels. The reduced secretory response to VIP when zinc or copper was present in the bathing media also indicated that the cAMP dependent chloride secretion was attenuated by zinc and copper. However, in our previous study we found that zinc also inhibited the calcium dependent carbachol induced secretion (Carlson et al., 2006). In neuronal research, it has been demonstrated that both zinc and copper are able to block Ca
channels (Castelli et al., 2003; Magistretti et al., 2003). Consequently, the third possible mechanism behind the reduced secretion caused by zinc and copper may be blockage of the basolateral K
and/or Ca
channels. The fourth hypothetically mode of action of zinc and copper is that these cations are able to alter the activity of membrane bound enzymes e.g. adenylyl cyclase which catalyses the intracellular cAMP synthesis. This mechanism has been documented for zinc in neuronal cells (Klein et al., 2002). It is not possible from the current study to predict if the attenuating effect of zinc and copper was due to inhibition of basolateral receptors, inhibition of basolateral membrane enzymes or blockage of basolateral ion channels or a combination of these. Furthermore, it is not clear if the differences between the effect of zinc and copper can be ascribed to different affinities or may be due to different mode of actions. Consequently, these mechanisms should be studied further in the future.

There was a large variation in growth rates between piglets and no statistical differences were seen. The mean feed intake did not match the corresponding growth rates and it is our experience that estimates on feed intake in weaned piglets are rather imprecise due to the relatively high feed spillages that occurs when piglets spend much time manipulating the feed and at the same time only consume very small amounts. Feeding high concentrations of ZnO is well recognized to have growth promoting effects in weaned pigs with the most pronounced effect in the second week after weaning (Case and Carlson, 2002; Hill et al., 2000). Consequently, the lack of effect of extra dietary zinc on growth in the present study may be due to the short experimental period. Accordingly, it may be speculated that during the first days after weaning high concentrations of dietary zinc mainly results in a higher intestinal resistance against diarrhoea inducing bacteria and subsequently, improved pig health results in increased feed intake and increased weight gain. In agreement with our previous studies, the piglets that were offered the ZnO2500 diet 5 to 6 d after weaning responded with a rise in zinc status in terms of increased zinc, AP and MT in plasma and tissues, compared with piglets offered the ZnO100 diet (Poulsen et al., 1995; Feng et al., 2006; Carlson et al., 2007). The plasma zinc concentration in the ZnO2500 group was similar to the concentration in pigs fed 2000 mg zinc/kg for 7 d post weaning (Buff et al., 2005) or 14 d post weaning (Martinez et al., 2004). The plasma zinc concentration in the ZnO100 group was much lower than concentrations (0.6–0.7 mg/l) in plasma from pigs fed similar quantities of zinc in other studies (Martinez et al., 2004; Buff et al., 2005; Carlson et al., 2007). This may indicate a very low zinc status of the piglets in the present study. The low zinc status may be associated with the weight loss of these piglets. However, plasma copper concentrations and haematocrit values were similar for the two dietary groups. Plasma copper concentrations were within the same levels as found by Creech et al. (2004). The similar haematocrit values between the two dietary groups may indicate that fluid balance was not affected by the dietary treatments. The plasma AP activity tended to increase with the high dietary zinc concentration, but for both groups the activity was within the same level as found in weaned piglets fed 80–230 mg zinc/kg DM (Creech et al., 2004). The liver MT concentration of the Zn2500 group was almost twofold the Zn100 group. Carlson et al. (1999) found that the MT concentration in liver increased about threefold when pigs were fed 3000 mg zinc/kg DM for 7 d. The only zinc status indicator that did not respond to the dietary zinc treatments was mucosa MT concentrations, which is in contrast to MT mRNA measured in mucosa 5 to 7 d after weaning (Carlson et al., 2007). This may confirm that MT gene expression does not always imply the presence of the MT protein (Vasconcelos et al., 2002). Furthermore, the lacking response to dietary zinc on mucosal MT in the present study and the increased mucosal MT, when piglets were fed a similar diet for 1 or 2 weeks after weaning (Carlson et al., 1999; Martinez et al., 2004), may indicate that piglets should be fed the ZnO2500 diet for more than 5 to 6 d to increase mucosal MT protein concentrations. One of the aims of the present study was to study if zinc status of the piglets affected the effect of zinc on provoked secretion from intestinal epithelium in vitro. The results show interactions between dietary zinc and zinc in vitro on the 5-HT response. Apparently this interaction was a consequence of a reduced secretory response to 5-HT in the control chambers when the piglets were fed the ZnO2500 diet compared with the ZnO100 diet which is in accordance with a previous study (Carlson et al., 2004). Consequently, these results may implicate that pigs with a high zinc status after weaning are less sensitive to diarrhoea inducing bacteria, but if zinc status for some reason is low, it may be possible to reduce diarrhoea symptoms by a quick increase in plasma zinc concentrations. The present study did not indicate any effect of piglet initial zinc status on the VIP induced secretion. The different influence of initial zinc status on the 5-HT and VIP induced secretion may be ascribed to the different signalling pathways of these secretagogues. VIP’s function is to increase intracellular cAMP levels resulting in chloride secretion, whereas 5-HT induce chloride secretion through a variety of pathways of which some so far unspecified, may be affected by initial epithelial zinc status.

It is concluded that zinc at the basolateral side of piglet small intestinal epithelium attenuated 5-HT and VIP induced secretion in vitro. Copper reduced the secretagogue-induced secretion to a larger extent than zinc; however, copper also disturbed the basal ion transport. Therefore it is unclear if the effect of copper was a toxic effect on a physiological response. The effect of zinc in vitro was reduced when the piglets had access to a diet containing 2500 compared with 100 mg zinc/kg from weaning until slaughter at 5 to 6 d after weaning. These results may implicate that dietary zinc should be absorbed to reduce post-weaning diarrhoea in piglets in vivo.
References


