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Zinc is involved in regulation of secretion from intestinal epithelium in weaned piglets ☆

D. Carlson [a,*], J. Sehested [a], Z. Feng [b], H.D. Poulsen [a]

[a] *Department of Animal Health, Welfare and Nutrition, Faculty of Agricultural Sciences, Research Centre Foulum, University of Aarhus, P.O. Box 50, 8830 Tjele, Denmark*
[b] *Ministry of Agriculture Feed Industry Centre, Beijing, China*

Abstract

The objective was to study the effect of serosal zinc (Zn) on intestinal chloride (Cl^-) secretion *in vitro* by the Ussing chamber technique. The secretagogues used to stimulate Cl^- secretion were serotonin (5-HT), vasoactive intestinal polypeptide (VIP) and forskolin (FSK). In addition correlations between organ and plasma Zn levels vs. the responses to 5-HT were studied. The results revealed an attenuating effect of serosal Zn on the secretory response to 5-HT, VIP and FSK. Furthermore, negative correlations between secretory responses to 5-HT vs. ADG and plasma Zn concentrations were found, whereas the responses to 5-HT did not correlate to the Zn concentration in liver or intestinal mucosa. It is suggested, that dietary Zn reduces diarrhoea directly through a regulatory role of serosal Zn on Cl^- secretion and indirectly by improving the nutritional status, which may stabilize the function of the intestinal epithelium.

Keywords: Ussing chamber; Serotonin (5-HT); Vasoactive intestinal polypeptide (VIP); Forskolin (FSK)

1. Introduction

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 pathogenic bacteria secrete enterotoxins in the intestinal lumen the neurotransmitter 5-HT is released from enterochromaffin cells located in the intestinal epithelium and a variety of receptors at the epithelial cells is activated resulting in chloride (Cl^-) secretion (Skadhauge et al., 1997). Furthermore, 5-HT induces the release of other neurotransmitters (e.g. VIP) from enteric nerve endings which elevates intracellular messengers (e.g. cAMP) followed by Cl^- secretion (Barrett and Keely, 2000). We have previously shown reduced epithelial responses to 5-HT *in vitro* when piglets were fed 2500 ppm of Zn compared to 100 ppm for 5 days after weaning (Carlson et al., 2004). Recently, we found a similar attenuating effect of Zn, on the response to 5-HT, VIP and carbachol, when 0.023 mM of Zn was added directly to the bathing media (Carlson et al., 2006). Similar *in vitro* studies with rat intestinal epithelium by Hoque et al. (2005) showed that 1 mM

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* Corresponding author. Tel.: +45 89 99 13 88; fax: +45 89 99 13 78.
E-mail address: dorthe.carlson@agrsci.dk (D. Carlson).

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serosal Zn reduced Cl^- secretion evoked by forskolin (FSK).

This study aimed to elucidate the effect of serosal Zn on provoked ion secretion *in vitro* by use of the Ussing chamber technique. The secretagogues used to provoke Cl^- secretion were serotonin (5-HT), vasoactive intestinal polypeptide (VIP) and forskolin (FSK). Furthermore, the correlation between the secretory capacity of the intestinal epithelium (measured as the 5-HT response *in vitro*) vs. the organ and plasma Zn concentrations of piglets was estimated. The presented data are obtained in two identical designed experiments.

2. Material and methods

Both studies included 24 piglets that were weaned and allocated to two different diets at 28 days of age. The two diets were wheat-, barley- and soybean meal based diets supplied with either 100 or 2500 ppm of dietary Zn (from ZnO). At 5 or 6 days after weaning, individual blood samples were taken at 08:00 h and subsequently, the piglets were slaughtered at 09:00 or 13:00 h. The euthanasia procedure included stunning with a bolt gun and exsanguination. The piglets were weighed at the day of weaning and at the day of slaughter.

Immediately after the piglets were killed, 30 cm of the middle of the small intestine was removed and placed in an oxygenated and phosphate-buffered Ringer solution at room temperature. For Zn measurements another 100 cm of small intestine (located proximal to the first site) was washed with cooled saline and the intestinal mucosa layer was scraped of. In addition, the liver from each piglet was collected. Plasma, mucosa and liver were analysed for Zn by atomic absorption spectrophotometry (Unicam SP9, Philips, Cambridge, UK).

2.1. Measurement of electrophysiological parameters

Within 15 min after killing, the epithelium was stripped of the muscle layers and mounted in Ussing chambers (WPI, Sarasota, Fl, USA) in 3 and 4 replicates for exp 1 and 2, respectively. The conditions of the Ussing chamber experiments were analogous to the conditions described by Carlson et al. (2004). The bathing media (a Ringer solution) were replaced 10 min after mounting the tissue. Thereafter, the tissues equilibrated for 20 min, before 0.023 mM of $ZnSO_4$ was added at the serosal side to 2 chambers (SZn) and a similar volume of water (200 µl, control) was added at the serosal side to 1 chamber (exp 1) or 2 chambers (exp 2). Twenty minutes

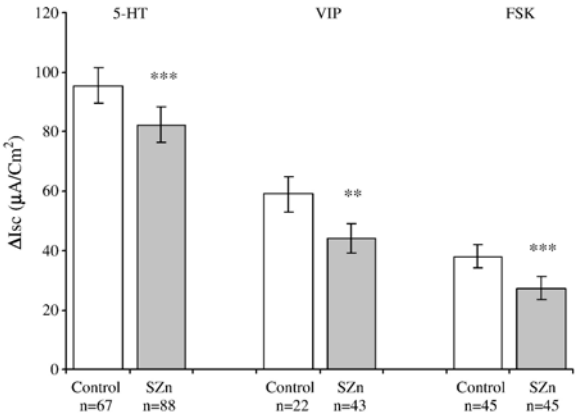

Fig. 1. The effect of Zn in the serosal bathing media (SZn) on changes in short circuit current (ΔIsc) induced by 0.1 mmol/l of 5-HT, 0.1 µmol/l Vasoactive Intestinal Polypeptide (VIP) and 10 µM forskolin (FSK) in piglet small intestinal epithelium. White bars represent a Zn free bathing media and black bars represent a serosal Zn concentration of 0.023 mmol/l. Values are least square means ± SEM, N=47 piglets for 5-HT and N=24 and 23 piglets for VIP and FSK, respectively, n=number of epithelial tissues per treatment. ** and *** indicate that SZn differs significantly from the control (**$p \leq .01$ and ***$p \leq .001$).

subsequently, 0.1 mM of 5-HT (Sigma, Copenhagen, Denmark) was added at the serosal side to all chambers followed 20 min later by 0.1 µM of VIP (Calbiochem, Albertslund, Denmark) (exp 1) or 10 µM FSK (Sigma, Copenhagen, Denmark) (exp 2) at the serosal side. The response (ΔIsc, change in short circuit current) to 5-HT, VIP and FSK was calculated by subtracting the basal Isc before addition from the peak Isc after addition of the secretagogue.

2.2. Statistical analysis

Statistical analysis was carried out by the MIXED procedure in SAS (Littel et al., 1996) with the fixed effects of dietary Zn treatment (100, 2500 ppm), *in vitro* Zn treatment (control, SZn) and the interaction between them. Litter and interactions between litter and pig were included in the model as random effects. Results are presented as least square means ± SEM with N=number of piglets and n=number of epithelial tissues. Effects were considered significant at $p \leq 0.05$. The pair-wise comparison procedure in SAS was used to separate the least square means.

The CORR procedure in SAS was used to test correlations between the secretory responses to 5-HT versus the average daily gain (ADG), plasma, mucosa and liver Zn concentrations, respectively. Least square means for ADG, plasma Zn, mucosa Zn and liver Zn