Intestinal epithelial responses to *Salmonella enterica* serovar Enteritidis: Effects on intestinal permeability and ion transport

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ABSTRACT *Salmonella* infection of chickens that leads to potential human foodborne salmonellosis continues to be a major concern. Chickens serve as carriers but, in contrast to humans, rarely show any clinical signs including diarrhea. The present investigations aimed to elucidate whether the absence of diarrhea during acute *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) infection may be linked to specific changes in the electrophysiological properties of the chicken gut. Immediately after slaughter, intestinal pieces of the mid-jejunum and cecum of either commercial broiler or specific pathogen-free (SPF) chickens were mounted in Ussing chambers in 2 separate experimental series. Living *Salmonella* Enteritidis (3×10^9) or *Salmonella* Enteritidis endotoxin (20 mg/L), or both, were added to the mucosal side for 1 h. In both experimental series, the *Salmonella* infection decreased the trans-epithelial ion conductance G_t ($P < 0.05$). In the jejunum of SPF chickens, there was also a marked decrease in net charge transfer across the epithelium, evidenced by decreased short-circuit current (I_{sc}, $P < 0.05$). Interestingly, the mucosal application of *Salmonella* endotoxin to the epithelial preparations from jejunum and cecum of SPF chicken had an effect similar to living bacteria. However, the endotoxin had no additional effect on the intestinal function in the presence of bacteria. The decreasing effect of *Salmonella* and or its endotoxin on G_t could be partly reversed by serosal addition of histamine. To our knowledge, this is the first study to address the functional response of native intestinal epithelium of chicken to an in vitro *Salmonella* infection. For the first time, it can be reported that intestinal ion permeability of chicken decreases acutely by the presence of *Salmonella*. This type of response could counteract ion and fluid secretion and may thus, at least in part, explain why chickens do not develop overt diarrhea after *Salmonella* infection.

Key words: chicken, diarrhea, intestinal permeability, endotoxin, *Salmonella* Enteritidis

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INTRODUCTION

Pathogenic enteric bacteria such as *Salmonella* are a significant health problem worldwide. Infections with this pathogen are the most frequent cause of foodborne outbreaks of gastroenteritis in adults and children (Stutman, 1994). *Salmonella* are a major problem to the poultry industry because chickens are regarded as the main source for human infections. They are considered as asymptomatic carriers, shedding the bacteria in feces without any clinical signs (Van Roekl, 1965). In adult birds, some serovars become localized in the reproductive tract (Barrow and Lovell, 1991). This epidemiological background may result in the entry of *Salmonella* into the human food chain (Van Roekl, 1965).

Some strains of *Salmonella* are capable of stimulating fluid secretion in ligated rabbit ileal loops, suggesting a possible role for enterotoxin (Giannella et al., 1973). It has been shown that severe epithelial damage occurs in several invasive and cytotoxin-producing bacteria (e.g., *Salmonella*; Giannella et al., 1977; Giannella, 1979). Epithelial lesions are observed after *Salmonella* Enteritidis infection of broiler chickens but are very moderate compared with those in mammals (Porter and Holt, 1993). Several potential virulence factors of *Salmonella* Enteritidis may contribute to infection and intestinal mucosal damage (Kwag et al., 2008). These factors include epithelial invasion, synthesis of an enterotoxin, and induction of an inflammatory response; however, the exact mechanisms by which *Salmonella* causes mucosal damage are not well understood (Mehta et al., 1998).

Some enteric pathogens have been shown to alter the permeability of the paracellular pathway by interfering with intercellular tight junctions of the intestinal epi-

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thelium (Yu and Yang, 2009). The paracellular leak may be evident even in the absence of histologic damage and contribute to disturbance of selective intestinal transport (e.g., toxin absorption) and diarrhea (Troeger et al., 2009). The functional disruption of tight junctions can be measured in vitro as an increase in transepithelial electrical conductance (G_t; Berkes et al., 2003). It has been shown in *Salmonella*-infected pigs that the intestinal function can be altered despite the absence of clinical signs (Berkes et al., 2003; Aschenbach et al., 2007).

So far, there have been no investigations focusing on functional aspects of the chicken gut, following a clinically asymptomatic *Salmonella* carriage. However, investigations on altered permeation pathways, as well as the responsiveness of the intestinal epithelium to prosecretory stimuli like, for example, histamine are important to characterize functionally the impact of *Salmonella* on the intestinal epithelial lining. Furthermore, *Salmonella* infection may induce segmental differences in secretion and absorption that do not lead to diarrhea but induce large shifts of solute and water movements between intestinal segments.

Consequently, the present investigations aimed to functionally characterize the role of intestinal epithelial cells in 2 different intestinal segments of 2 different chicken breeds in the host response to an in vitro infection with *Salmonella* Enteritidis.

MATERIALS AND METHODS

Birds and Feeding

Two different experiments were done. In the first experiment, specific pathogen-free (**SPF**) chickens (VALO Lohmann, male and female) were used at 16 wk of age, weighing 1.5 kg (n = 10). In the second experiment, broiler chickens (male and female) 6 wk of age were used, weighing 2.0 kg (n = 10). The SPF birds reflected more the genetic background of laying birds, whereas commercial broilers were somewhat different because they are bred for rapid growth. Including both genetic lines reflects the practical situation of poultry production. The broilers were purchased from a local commercial farm. The birds (broiler and SPF chicken) were housed on wood shavings and were fed a commercial diet (Mischfutterwerk Marchtrenk Likra Tierernährung GmbH & Co. KG, Marchtrenk, Austria). The diet contained 21% CP, 5.3% fat and oil, 3.1% crude fiber, 6.5% crude ash, and 1.2% lysine. The birds were provided with their diets and water ad libitum during the experiments. The animal experiments were discussed and approved by the institutional ethics committee under license number GZ 68.205/0006-II/3b/2011.

Preparation of Intestinal Epithelium and Ussing Chamber Setup

The preparation of epithelia and mounting in Ussing chambers was previously described (Awad et al., 2007). Immediately after killing, the mid-jejunum and cecum were harvested from the birds and placed into ice-cold buffer solution (see below) oxygenated with carbogen (95% O_2/5% CO_2). The intestinal segments were opened along the mesenteric border and washed free of intestinal content with buffer solution at 4°C. The underlying serosal layer was stripped off, and the epithelial sheets were mounted in Ussing chambers. Epithelial sheets had an exposed serosal area of 1.1 cm^2 and were incubated with 12 mL of buffer solution on their mucosal and serosal sides under short-circuit conditions. Up to 12 chambers were used for each bird.

Buffer Solutions

The buffer solution used for washing, transport, and incubation of epithelia contained the following chemicals (Sigma-Aldrich Chemie GmbH, in mmol/L): NaCl, 115; KCl, 5; $CaCl_2$, 1.5; $MgCl_2$, 1.2; NaH_2PO_4, 0.6; Na_2HPO_4, 2.4; L-glutamine, 1; Na-D/L-lactate, 5; HEPES-free acid, 10; $NaHCO_3$, 25; and mannitol, 10 (320 ± 5 mosmol/kg; pH 7.4). The serosal bathing solution contained 10 m*M* glucose and was balanced osmotically on the mucosal side with 10 m*M* mannitol. The incubation medium was continuously gassed with carbogen, and the temperature of the mixture was kept at 38°C by thermostated water jackets. Continuous oxygenation provided recirculation of the incubation solutions by means of a gas lift.

Electrophysiological Measurements

Before mounting of epithelia, junction potential and fluid resistance were determined by a computer-controlled voltage clamp device (Ing.-Büro für Mess-und Datentechnik, Aachen, Germany) for later automatic correction of electrophysiological measurements. The potential difference (***PD***) was measured using KCl-agar bridges connected to Argenthal electrodes (Mettler Toledo, Columbus, OH), and the *PD* was short-circuited through Ag-AgCl electrodes using a voltage clamp corrected for fluid resistance to obtain measurements of short-circuit current (I_{sc} in $\mu A/cm^2$). The tissues were first incubated under open-circuit conditions for 20 min and then voltage-clamped by fixing the voltage at 0 mV. Thereby, I_{sc} provides a direct measure for the electrical sum of all ions transported across the epithelium. Tissue conductance (G_t in mS/cm^2) was determined by measuring the changes in transepithelial potential difference upon short bipolar current impulses ($G_t = \Delta I/\Delta PD$).

The basal measurements of I_{sc} and G_t were taken after a stabilization period of 30 min (low/or no fluctuation of the measurements). *Salmonella* Enteritidis (ATCC 13076) was routinely grown in Lennox L Broth Base (Invitrogen, Carlsbad, CA) (LB)-broth at 37°C for 24 h in a shaking incubator. *Salmonella* cfu were determined from each suspension by serial dilutions in duplicate using LB agar. *Salmonella* suspensions were stored at −80°C by adding 2 mL of 40% glycerol/10

mL of LB broth. For use in the Ussing Chamber, *Salmonella* suspensions were centrifuged for 5 min at 4,000 $\times$ *g* at 4°C. The pellets were washed 3 times by Ussing buffer solution, resuspending the pellet, and centrifugation of the resuspension at the same conditions mentioned above. Finally, the pellets were resuspended in Ussing buffer and used in the Ussing chamber at dose (3×10^9 cfu/mL).

When the tissue had stabilized, *Salmonella* Enteritidis (3×10^9) or *Salmonella* Enteritidis endotoxin (20 mg/L; L6011, Sigma-Aldrich GmbH, Vienna, Austria), or both, were added to the mucosal side, and I_{sc} and G_t were monitored for 1 h. Simultaneously control tissues were incubated without *Salmonella* or *Salmonella* endotoxin additions (or both) to obtain data for time-dependent changes in I_{sc} and G_t. The basal I_{sc} and G_t represent the actual values before each addition. The effects of *Salmonella* or endotoxin application to the mucosal side on the electrical variables are given as the changes in G_t or I_{sc} (ΔG_t or ΔI_{sc}), which were calculated for each tissue as the difference between the G_t or I_{sc} at a given time after challenge with *Salmonella* or endotoxin (or both) and the basal steady state value of G_t or I_{sc}. Histamine, a neural and immune mediator that commonly elicits secretion, was tested for its influence on I_{sc} and G_t after infection. Histamine was applied basolaterally after 1 h of incubation with *Salmonella* or endotoxin, or both (or the corresponding time point in control tissues), and the tissues were further incubated for at least 15 min.

To investigate if a *Salmonella* infection has a similar effect on broilers, a second experimental series was conducted with broilers (6 wk of age). The general setup for both experiments was identical. Electrophysiological variables, I_{sc} and G_t, of the intestinal epithelium were recorded throughout both experiments.

Statistical Analysis

Data are presented as means with SEM. After testing for normality (Kolmogorov-Smirnov's test), statistical analysis for significant differences between 2 groups was performed using Student's *t*-test. Statistical differences at probability values of 0.05 ($P < 0.05$) were considered significant. Multiple groups were compared using the one-way ANOVA, and statistically different means ($P < 0.05$) were further separated using least significant difference and Duncan's multiple range test. Time-dependent changes within groups were assessed by repeated-measures ANOVA. All tests were performed using appropriate software (PASW statistics 17, SPSS, Chicago, IL).

RESULTS

Application of Salmonella or Endotoxin, or Both

In the jejunum of SPF chickens, luminal addition of *Salmonella* Enteritidis induced a prompt and marked drop in G_t ($P < 0.01$; Figure 1). A comparable drop in G_t was also induced by application of *Salmonella* endotoxin and by a combined application of live *Salmonella* and *Salmonella* endotoxin ($P < 0.01$; Figure 2). The latter implies that endotoxin was as effective as living *Salmonella* to alter intestinal function but had no additional effect when *Salmonella* was already present. The decrease in G_t coincided with a decrease in net charge transfer across the epithelium, evidenced by decreased I_{sc} after *Salmonella* or endotoxin application or combined application of *Salmonella* and endotoxin ($P < 0.01$; Figure 2). Table 1 lists the absolute and relative changes in G_t and I_{sc} from baseline values in comparison with untreated control tissues. The absolute and relative changes in G_t and I_{sc} were not different between the *Salmonella*, endotoxin, or *Salmonella* and endotoxin-treated epithelia. However, all 3 groups had significantly larger decreases in G_t and I_{sc} compared with the untreated control tissues ($P < 0.05$).

Table 2 shows that the decreasing effect of *Salmonella* or its endotoxin, or both, on G_t was numerically demonstrated in the cecum of SPF chickens without statistical significance. However, the decrease of G_t by in vitro *Salmonella* infection was significant in the jejunum and cecum of broiler chickens ($P < 0.05$, Tables 3 and 4). In the jejunum of broiler chickens, a trend for a larger decrease in I_{sc} was observed for *Salmonella*-infected tissues compared with untreated control tissues. The combined application of *Salmonella* and *Salmonella* endotoxin in the cecum of SPF chickens also induced a decrease in I_{sc} that was different from untreated tissues. However, decreases in I_{sc} could not be observed when *Salmonella* was applied alone in the cecum of either SPF (Table 2) or broiler chickens (Table 4).

Application of Histamine

From studies in mammals, it is known that histamine receptors are widely distributed in the gastrointestinal system and are involved in the stimulation of epithelial secretion. Although changes in receptor population and responsiveness of tissues to histamine are known to occur under some disease conditions, there is no information regarding the effect of histamine on *Salmonella*-infected tissues in chickens. The responsiveness of the *Salmonella*-infected intestinal tissues to histamine was therefore studied. In the jejunum of broiler chickens, serosal histamine application after *Salmonella* infection led to a prompt increase in G_t that was different from the numerical decrease in the untreated control tissues ($P < 0.01$; Table 3). A similar response was observed in the jejunum and cecum of SPF chickens (i.e., G_t numerically increased after histamine application when *Salmonella* or endotoxin, or both, were applied beforehand). The latter increases in G_t tended to be different from the negative values in the untreated control tissues ($P \leq 0.1$; Tables 1 and 2) and reversed the *Salmonella*-induced decreases of G_t (Tables 1 and 2). These results suggest that *Salmonella* infection induced responsive-

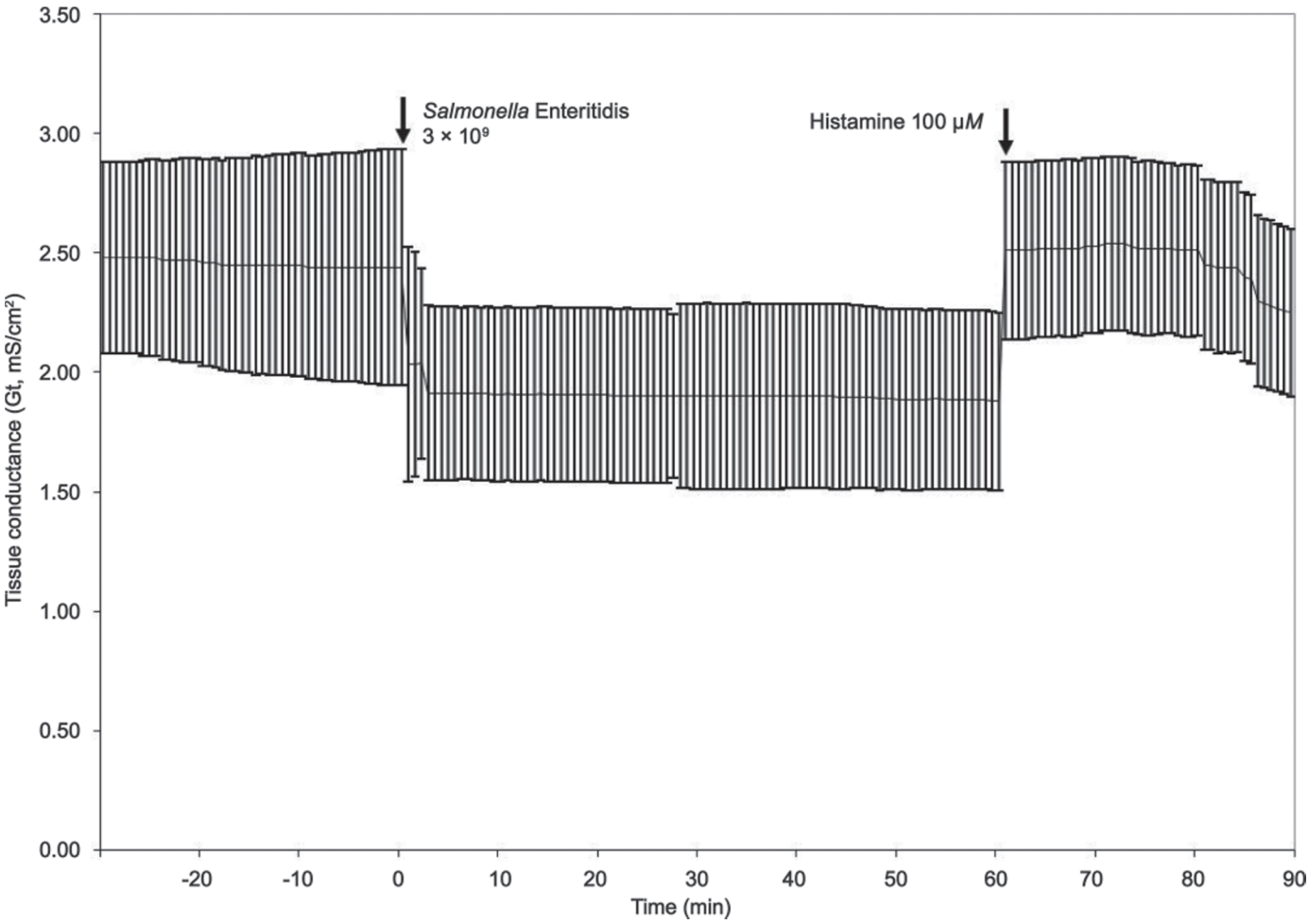

Figure 1. Time course of the tissue permeability (G_t) of jejunal epithelial sheets of specific pathogen-free chickens after exposure to living *Salmonella* Enteritidis on the luminal side and histamine on the serosal side. Epithelial sheets were incubated in Ussing chambers. Data are given as means ± SEM [n = 10 (number of experiments for each treatment)].

ness to histamine in these tissues. In contrast to the rather consistent pattern of changes in G_t, changes in I_{sc} were very variable and never significantly different between groups (Tables 1, 2, 3, and 4). These findings in chicken do not coincide with the investigations in mammals where histamine induced increases in I_{sc} in all tissues studied so far (Ahrens et al., 2003; Aschenbach et al., 2003; Schultheiss et al., 2006).

DISCUSSION

Salmonella is an important cause of food-borne diseases in humans and is also an important cause for gastrointestinal disorders in other mammals (Stutman, 1994). The most prominent clinical sign is diarrhea caused by *Salmonella* enterotoxins that initiate fluid secretion into the intestinal lumen (Giannella et al., 1973). Furthermore, severe epithelial damage can occur due to invasion and cytotoxin production by *Salmonella* (Giannella, 1979; Giannella et al., 1977). The present study was designed to address the question whether functional characteristics of the chicken intestine may explain why this species is better protected against the occurrence of *Salmonella*-induced diarrhea than mammals and rather serves as an inapparent carrier.

The intestinal mucosa acts as a defensive barrier, which selectively permits absorption of nutrients while preventing access by pathogens (Zareie et al., 2001; Bischoff and Krämer, 2007; Yu and Yang, 2009; Smith et al., 2011; Xiao et al., 2011). This defensive barrier is organized according to the anatomical layers of the mucosa (Wallace and Granger, 1996), with the intact epithelium representing the most important physical barrier component for selective and nonselective permeation. The transmural ion conductance is a measure commonly used to assess the permeability of this barrier in Ussing chamber experiments. Bertelsen et al. (2003) reported that *Salmonella* Typhimurium infection rapidly increases Cox-2 expression in human intestinal tissue, which is responsible for an increased epithelial ion transport that underlies secretory diarrhea associated with *Salmonella* infection. Enteric *Salmonella* infection is accompanied by inflammation and diarrhea, but little is known about its effects on intestinal epithelial physiology. *Salmonella* can induce changes in the epithelium and alter intestinal function. Therefore,

Table 1. The transmural conductivity (G_t) and short-circuit current (I_{sc}) of isolated jejunal mucosa of specific pathogen-free chickens in response to *Salmonella* or endotoxin application, or both, and subsequent histamine application

Item[1]	*Salmonella*	Endotoxin	*Salmonella* + endotoxin	Control	SEM[2]	P[3]
Basal G_t (mS/cm^2)	2.48	2.53	3.28	2.45	0.25	0.620
Basal I_{sc} (μEq/cm^2·h)	2.30	3.90	3.00	3.75	0.32	0.267
Salmonella or endotoxin application (or both)[4]						
ΔG_t (mS/cm^2)[5]	−0.53^{ab}	−0.62^{a}	−0.69^{a}	−0.10^{b}	0.08	0.043
ΔG_t (%)[6]	−22.18^{a}	−23.85^{a}	−20.00^{a}	−4.92^{b}	3.41	0.001
ΔI_{sc} (μEq/cm^2·h)[7]	−1.60^{ab}	−2.00^{a}	−2.20^{a}	−0.25^{b}	0.30	0.040
ΔI_{sc} (%)[8]	−65.00^{a}	−48.48^{a}	−77.78^{a}	−7.64^{b}	8.77	0.005
Histamine application[9]						
ΔG_t (mS/cm^2)	+0.55	+0.16	+0.26	−0.07	0.10	0.154
ΔG_t (%)	+38.24	+8.93	+12.87	−0.10	5.66	0.092
ΔI_{sc} (μEq/cm^2·h)	+0.13	+0.38	+0.13	−0.50	0.14	0.137
ΔI_{sc} (%)	+22.22	+14.58	+12.50	−8.85	6.08	0.184

[a,b]Values within one row that do not share a common letter are different ($P < 0.05$; Duncan's test).

[1]I_{sc} or G_t at time zero is the basal value before addition *of Salmonella* or endotoxin (or both).

[2]Data are arithmetic means and pooled SEM [n = 10 (number of experiments for each treatment)].

[3]Probability values of 0.05 ($P < 0.05$).

[4]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 60 min after application.

[5]ΔG_t = (G_t at time t) − (G_t at time zero).

[6]ΔG_t% = [(G_t at time t) − (G_t at time zero)]/G_t at time zero.

[7]ΔI_{sc} = (I_{sc} at time t) − (I_{sc} at time zero).

[8]ΔI_{sc}% = [(I_{sc} at time t) − (I_{sc} at time zero)]/I_{sc} at time zero.

[9]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 5 min after application.

the present investigations aimed to characterize the changes of intestinal epithelial cells after infection with *Salmonella* Enteritidis.

In the present studies, *Salmonella* induced a prompt decrease in the transmural conductance that proved significantly larger than the time-dependent decrease in conductance in the untreated control tissues in the jejunum and cecum of SPF chickens, in the jejunum of broiler chickens and, as a trend, also in the cecum of broiler chickens. This means that all tested intestinal segments became tighter to passive ion permeation within a few minutes after *Salmonella* infection. In the jejunum and cecum of SPF chickens, we further demonstrated that the effect of *Salmonella* infection on G_t can be reproduced by adding only *Salmonella* endotoxin. Considering also the rapid onset of the decrease in G_t, the latter suggests that invasion of *Salmonella* may not be required to elicit the effect; the contact of the epithelium with endotoxin is already sufficient.

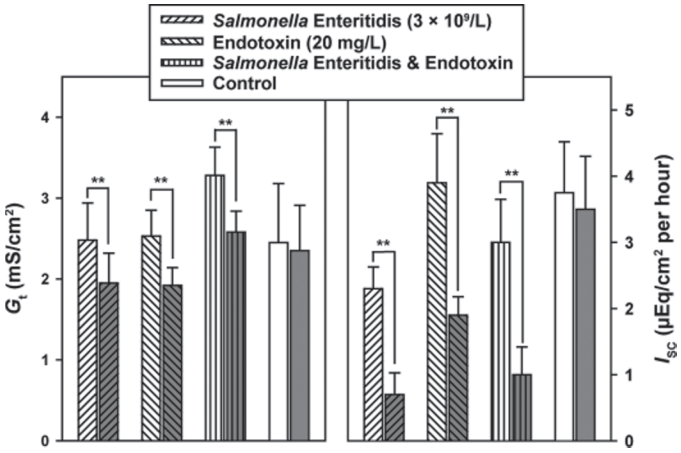

Figure 2. Effect of luminal *Salmonella* Enteritidis or *Salmonella* Enteritidis endotoxin (or both) on the permeability (G_t) and short-circuit current (I_{sc}) of isolated jejunal epithelial sheets from 16-wk-old specific pathogen-free chicken. Epithelial sheets were incubated in Ussing chambers. Living *Salmonella* Enteritidis or *Salmonella* endotoxin (or both) were applied to the luminal side. White columns represent basal values before additions, whereas gray columns represent values 1 h after addition of *Salmonella* or endotoxin (or both) as indicated by the hatching pattern. Data from simultaneously incubated epithelia without treatment served as controls. Data are given as means + SEM [n = 10 (number of experiments for each treatment)]. **Asterisks mark significant differences ($P < 0.01$).

In a previous study, we had demonstrated that feeding *Salmonella* endotoxin over 14 d decreases tissue conductance in the colon of pigs (Aschenbach, et al., 2003). In the same experiment, however, bilateral application of *Salmonella* endotoxin to isolated colonic sheets did not have a quick effect on G_t, either in control or in endotoxin pre-fed pigs (Aschenbach, et al., 2003). This may suggest that the mammalian intestine is, in principle, able to react to endotoxin in the same way as the chicken intestine but may require prolonged stimulation.

Salmonella endotoxin is recognized via Toll-like receptors TLR4 and TLR2 (Tang et al., 2006), which are both present on intestinal epithelial cells (Hanson et al., 2011). Although signaling via TLR4 is the classical way of endotoxin signaling and leads to increased conductance in mammalian intestinal epithelial cells upon endotoxin stimulation (Lotz et al., 2006; Albin et al., 2007), TLR2 has the opposite effect. It preserves the epithelial barrier and decreases conductance (Cario et al., 2004; Hanson et al., 2011). Consequently, the ability of the chicken intestine to react to *Salmonella* or *Salmonella* endotoxin exposure with a prompt decrease in passive ion permeation may be seen in an enhancement of TLR2 relative to TLR4 signaling in this spe-

Table 2. The transmural conductivity (G_t) and short-circuit current (I_{sc}) of isolated cecal epithelial sheets of specific pathogen-free chickens in response to *Salmonella* or endotoxin application, or both, and subsequent histamine application

Item[1]	*Salmonella*	Endotoxin	*Salmonella* + endotoxin	Control	SEM[2]	P[3]
Basal G_t (mS/cm^2)	16.97	14.54	17.30	13.94	1.78	0.896
Basal I_{sc} (μEq/cm^2·h)	17.60	10.00	23.40	9.00	2.62	0.166
Salmonella or endotoxin application, or both[4]						
ΔG_t (mS/cm^2)[5]	−3.13	−2.08	−4.76	−0.61	0.85	0.132
ΔG_t (%)[6]	−16.44	−15.65	−22.78	−3.40	5.13	0.108
ΔI_{sc} (μEq/cm^2·h)[7]	+3.60^b	−2.80^b	−6.20^a	+2.00^b	1.43	0.043
ΔI_{sc} (%)[8]	+12.99	−30.16	−33.35	+22.11	10.65	0.134
Histamine application[9]						
ΔG_t (mS/cm^2)	+1.03	+4.42	+0.73	−0.09	1.04	0.100
ΔG_t (%)	+8.88^{ab}	+25.03^a	+5.12^{ab}	−0.46^b	5.08	0.056
ΔI_{sc} (μEq/cm^2·h)	+2.60	−1.00	−4.20	+0.80	1.99	0.302
ΔI_{sc} (%)	+26.02^b	−7.17^{ab}	−41.46^a	+17.46^{ab}	11.65	0.057

^{a,b}Values within one row that do not share a common letter are different ($P < 0.05$; Duncan's test).

[1]I_{sc} or G_t at time zero is the basal value before addition of *Salmonella* or endotoxin (or both).

[2]Data are arithmetic means and pooled SEM [n = 10 (number of experiments for each treatment)].

[3]Probability values of 0.05 ($P < 0.05$).

[4]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 60 min after application.

[5]ΔG_t = (G_t at time t) − (G_t at time zero).

[6]ΔG_t% = [(G_t at time t) − (G_t at time zero)]/G_t at time zero.

[7]ΔI_{sc} = (I_{sc} at time t) − (I_{sc} at time zero).

[8]ΔI_{sc}% = [(I_{sc} at time t) − (I_{sc} at time zero)]/I_{sc} at time zero.

[9]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 5 min after application.

cies. Interestingly, an insufficient signaling via TLR4 can simultaneously explain the increased carrier status of chicken for *Salmonella* Enteritidis (Chaussé et al., 2011). The assumption of signaling via TLR2 would further suggest that similar G_t responses can be expected upon exposure to other bacteria because TLR2 is a molecular pattern-recognition receptor that recognizes the peptidoglycan components of bacterial cell walls (Zenhom et al. 2012). Consequently, it will have to be tested in future studies whether other bacteria or their cell wall components are also able to decrease G_t in the chicken intestine.

Passive ion permeation can occur either via ion channels localized in cell membranes (i.e., transcellularly) or via junctional complexes connecting neighboring cells (i.e., paracellularly; Pácha, 2000). An assessment of I_{sc} can be helpful to distinguish between the 2 possibilities. Under the present experimental conditions where the PD across the junctional complexes was clamped to 0 mV, the applied clamp current short-circuits the

Table 3. The transmural conductivity (G_t) and short-circuit current (I_{sc}) of isolated jejunal mucosa of broiler chickens in response to *Salmonella* application and subsequent histamine application

Item[1]	*Salmonella*	Control	SEM[2]	P[3]
Basal G_t (mS/cm^2)	4.13	2.04	0.27	0.140
Basal I_{sc} (μEq/cm^2·h)	2.83	3.78	0.34	0.176
Salmonella application[4]				
ΔG_t (mS/cm^2)[5]	−1.67	−0.15	0.16	0.014
ΔG_t (%)[6]	−35.49	−6.74	3.73	0.008
ΔI_{sc} (μEq/cm^2·h)[7]	−0.83	−0.67	0.21	0.203
ΔI_{sc} (%)[8]	−28.33	−16.11	7.26	0.051
Histamine application[9]				
ΔG_t (mS/cm^2)	+0.70	−0.08	0.25	0.002
ΔG_t (%)	+25.95	−3.22	10.50	0.020
ΔI_{sc} (μEq/cm^2·h)	+0.50	−0.33	0.29	0.171
ΔI_{sc} (%)	+12.50	−7.41	11.57	0.849

[1]I_{sc} or G_t at time zero is the basal value before addition of *Salmonella* or endotoxin (or both).

[2]Data are arithmetic means and pooled SEM [n = 10 (number of experiments for each treatment)].

[3]Probability values of 0.05 ($P < 0.05$).

[4]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 60 min after application.

[5]ΔG_t = (G_t at time t) − (G_t at time zero).

[6]ΔG_t% = [(G_t at time t) − (G_t at time zero)]/G_t at time zero.

[7]ΔI_{sc} = (I_{sc} at time t) − (I_{sc} at time zero).

[8]ΔI_{sc}% = [(I_{sc} at time t) − (I_{sc} at time zero)]/I_{sc} at time zero.

[9]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 5 min after application.

Table 4. The transmural conductivity (G_t) and short-circuit current (I_{sc}) of isolated cecal epithelial sheets of broiler chickens in response to *Salmonella* application and subsequent histamine application

Item[1]	*Salmonella*	Control	SEM[2]	P[3]
Basal G_t (mS/cm^2)	13.04	12.41	0.93	0.750
Basal I_{sc} (μEq/cm^2·h)	19.78	37.50	4.21	0.030
Salmonella application[4]				
ΔG_t (mS/cm^2)[5]	−1.17	−0.36	0.19	0.027
ΔG_t (%)[6]	−9.20	−2.11	1.44	0.008
ΔI_{sc} (μEq/cm^2·h)[7]	−4.33	−4.13	2.92	0.973
ΔI_{sc} (%)[8]	−11.74	−17.48	16.36	0.390
Histamine application[9]				
ΔG_t (mS/cm^2)	+0.53	+0.37	0.32	0.807
ΔG_t (%)	+4.68	+3.38	3.17	0.846
ΔI_{sc} (μEq/cm^2·h)	+0.17	−1.00	2.02	0.788
ΔI_{sc} (%)	+1.18	−21.06	9.51	0.320

[1]I_{sc} or G_t at time zero is the basal value before addition of *Salmonella* or endotoxin (or both).

[2]Data are arithmetic means and pooled SEM [n = 10 (number of experiments for each treatment)].

[3]Probability values of 0.05 ($P < 0.05$).

[4]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 60 min after application.

[5]ΔG_t = (G_t at time t) − (G_t at time zero).

[6]ΔG_t% = [(G_t at time t) − (G_t at time zero)]/G_t at time zero.

[7]ΔI_{sc} = (I_{sc} at time t) − (I_{sc} at time zero).

[8]ΔI_{sc}% = [(I_{sc} at time t) − (I_{sc} at time zero)]/I_{sc} at time zero.

[9]Values represent the absolute and relative changes of G_t and I_{sc} from 1 min before application to 5 min after application.

charge separation achieved by cellular ion channels, and I_{sc} provides an indirect measure for the transcellular charge separation (Clarke, 2009). Consequently, a simultaneous change of I_{sc} and G_t is indicative for an involvement of cellular ion channels in any observed G_t response. The coincidence of the decreases in G_t and I_{sc} after *Salmonella* infection or endotoxin application, or both, in jejunal tissues of SPF and (as a trend) broiler chickens, therefore, suggests that the closure of cellular ion channels, at least partly, contributed to the decreased jejunal G_t upon exposure to *Salmonella*, its endotoxin, or both. Except for the combined application of *Salmonella* and endotoxin in SPF chicken, similar decreases in I_{sc} were not observed for the cecum in SPF and broiler chickens. This may point to a predominant involvement of the paracellular pathway in the conductance changes observed in the cecum. However, further studies are necessary to precisely assess the quantitative contribution of the transcellular vs. paracellular pathways to conductance changes after *Salmonella* exposure in both types of tissue.

Irrespective of whether *Salmonella* exposure predominantly decreases the transcellular or paracellular conductance, either option may serve to explain a decreased proneness of the chicken intestine to *Salmonella*-induced diarrhea. Fluid secretion into the intestine is mostly initiated by the opening of cellular channels for Cl^- and K^+ (Schultheiss et al., 2006), and sustained by passive flux of ions and water through the paracellular pathway (Viswanathan et al., 2009). According to this concept, the closure of cellular Cl^- and K^+ channels and a closure of the paracellular pathway would both decrease the fluid outflow into the intestine and thus ameliorate diarrhea.

Salmonella infection of mammals is associated with increased release of histamine from mast cells, which contributes to the development of diarrhea (Aschenbach et al., 2003). In all intestinal tissues studied so far, the main effect of histamine was an increase in chloride secretion, which can be evidenced in Ussing chambers by an increased I_{sc} (Ahrens et al., 2003; Schultheiss et al., 2006). A similar mode of action was previously also shown for the ileum of broiler chickens (Collins et al., 2007). An unexpected finding of the present study was that such response of histamine on I_{sc} could not be observed in the tested tissues. However, histamine increased or tended to increase conductance in jejunal and cecal tissues from SPF chickens and jejunal tissues from broiler chickens tissues after *Salmonella* or endotoxin exposure. This shows that preexposure to *Salmonella* or endotoxin was required for the G_t response of the intestinal epithelium to this secretory mediator. In contrast, histamine was ineffective in the untreated control tissues. The precise reason for this missing response remains unclear at present and has to be subject to further studies.

In summary, the present results suggest that luminal *Salmonella* Enteritidis affects the intestinal epithelium of broiler and SPF layer chicken in the same way as endotoxin and downregulates ion permeability directly after exposure. This is in contrast to findings in pigs where endotoxin does not elicit an acute decrease in permeability (Aschenbach et al., 2003). This finding could explain why chicken do not experience overt secretory diarrhea when infected by this pathogen in contrast to pigs and other species, including humans. A reduced responsiveness of the chicken intestinal epithelium to the pro-secretory mediator histamine may further contrib-

ute to the absence of diarrhea after *Salmonella* infection of chickens. Consequently, the absence of diarrhea in *Salmonella* Enteriditis-infected chickens may be seen as a result of a differently regulated gut function rather than a general resistance to infection. The results suggest one possible explanation for lack of clinical signs and the concurrent proneness to persistent infection. The present study model would be helpful for further studies on *Salmonella* infection in chickens.

To date, aside from the data as discussed and presented in this manuscript, the involvement of pathogenic bacteria and their tremendous potential for modulating normal physiological response of the intestinal epithelium have received scant attention by avian researchers. The present investigation revealed that results regarding the epithelial response to infection cannot be simply transferred from the mammalian to the avian intestine. This opens a challenging area for future research with the aim to discover the molecular basis of the differential responses of the chicken intestine to pathogenic infections.

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