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Stimulation of Transepithelial Na^+ Current by Extracellular Gd^{3+} in *Xenopus laevis* Alveolar Epithelium

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Abstract. In the present study we investigated the effect of extracellular gadolinium on amiloride-sensitive Na^+ current across *Xenopus* alveolar epithelium by Ussing chamber experiments and studied its direct effect on epithelial Na^+ channels with the patch-clamp method. As observed in various epithelia, the short-circuit current (I_{sc}) and the amiloride-sensitive Na^+ current (I_{ami}) across *Xenopus* alveolar epithelium was downregulated by high apical Na^+ concentrations. Apical application of gadolinium (Gd^{3+}) increased I_{sc} in a dose-dependent manner (EC_{50} = 23.5 μM). The effect of Gd^{3+} was sensitive to amiloride, which indicated the amiloride-sensitive transcellular Na^+ transport to be upregulated. Benzimidazolyl-guanidin (BIG) and *p*-hydroxy-mercuribenzonic-acid (PHMB) probably release apical Na^+ channels from Na^+-dependent autoregulating mechanisms. BIG did not stimulate transepithelial Na^+ currents across *Xenopus* lung epithelium but, interestingly, it prevented the stimulating effect of Gd^{3+} on transepithelial Na^+ transport. PHMB increased I_{sc} and this stimulation was similar to the effect of Gd^{3+}. Co-application of PHMB and Gd^{3+} had no additive effects on I_{sc}. In cell-attached patches on *Xenopus* oocytes extracellular Gd^{3+} increased the open probability (NP_o) of *Xenopus* epithelial sodium channels (ENaC) from 0.72 to 1.79 and decreased the single-channel conductance from 5.5 to 4.6 pS. Our data indicate that *Xenopus* alveolar epithelium exhibits Na^+-dependent non-hormonal control of transepithelial Na^+ transport and that the earth metal gadolinium interferes with these mechanisms. The patch-clamp experiments indicate that Gd^{3+} directly modulates the activity of ENaCs.

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Key words: *Xenopus laevis* — Lung — Amiloride — Epithelial Na^+ channel — Gadolinium — Self-inhibition — Patch clamp

Introduction

Sodium absorption in epithelial tissues is described to be a two-stage process (Koefoed-Johnson & Ussing, 1958). Na^+ ions enter the cells passively across the apical membrane and follow the electrochemical gradient, which is maintained by the basolateral Na^+/K^+-ATPase. The ATPase extrudes Na^+ ions in exchange with K^+ ions with a ratio of 3:2. Intracellular potassium is recycled via K^+ channels in the basolateral membrane. The rate-limiting step for Na^+ uptake across epithelia is attributed to the highly selective epithelial Na^+ channels (ENaCs) in the apical cell membranes of various tissues, i.e., in frog skin, distal kidney nephron, lung and colon (Garty & Palmer, 1997).

Extra- and intracellular Na^+ concentrations have been shown to have a regulatory influence on transepithelial Na^+ transport and to activate these ENaCs. Fuchs, Larsen and Lindemann (1977) described a regulation of Na^+ channels, dependent on apical Na^+ concentrations ($[Na^+]_a$). The self-inhibition, in response to high extracellular Na^+ concentrations, described a reduction of sodium entry into the cells according to a decrease of conducting channels (Lindemann, 1984; Turnheim, 1991). In addition, the feedback inhibition of epithelial Na^+ channels resulted from an elevated level of intracellular $[Na^+]_i$ (Turnheim, 1991). These mechanisms were thought to prevent epithelial cells from Na^+ loading, and thus to protect cells from excessive changes of the intracellular ion composition and cell volume (Turnheim, 1991).

As yet these inhibitory mechanisms are far from being understood. The participation of protein kinase C (Frindt, Palmer & Windhager, 1996) and G protein-mediated action (Komwatana et al., 1998), as well as of cytoskeletal elements (Els & Chou, 1993) and the ubiquitin-protein ligase Nedd4 (Dinudom et al., 1998; Farr et al., 2000) are suggested for feedback inhibition. In contrast, selt-inhibition may be mediated primarily by apical sodium but also second messengers, like cytosolic Ca^{2+}, seem to be involved (Ling & Eaton, 1989). Recent studies demonstrated that self-inhibition could be inhibited, e.g., by proteases or sulfhydryl-reactive reagents when applied to the cell surface (Gilbertson & Zhang, 1998; Snyder, Bucher & Olson, 2000, Chraibi & Horisberger, 2002; Kellenberger, Gautschi & Schild, 2002).

The investigation of effects of lanthanides on ion conductances has a long tradition in electrophysiology (Sunano, 1982; Hamill & McBride, 1996). The rare earth metal gadolinium (Gd^{3+}), e.g., has been used for the detection of stretch-activated ion channels (SACs) (Caldwell, Clemo & Baumgarten, 1998; Reifarth, Clauss and Weber, 1999) and acid-sensing ion channels (ASICs) (Babinski et al., 2000; Allen & Atwell, 2002). Secondly, Gd^{3+} is suggested to be an agonist of the Ca^{2+} receptor (Ray & Northup, 2002) and recent studies evaluated the toxicity of gadolinium (Fuma et al., 2001). At present, Friis and Nielsen (2001) have exclusively reported that Gd^{3+} activates apical sodium channels in the frog skin. This effect was interpreted as Gd^{3+} interference with the intrinsic Na^+-feedback inhibition system.

In the present study we investigated transepithelial Na^+ absorption, the main driving force for liquid clearance of the alveolar airspace from *Xenopus* lung, and tested Gd^{3+} for its effect on ion transport in dissected *Xenopus* lung epithelia. Besides Ussing chamber experiments, we performed patch-clamp experiments in order to investigate whether the earth metal directly interacts with ENaCs.

Materials and Methods

Animals and Tissue Preparation

Adult females of the clawed frog *Xenopus laevis*, purchased from H. Kähler (Hamburg, Germany), were kept in tap water at room temperature. They were fed once a week with commercial fish food. Each frog was injected subcutaneously with 1 nmol adrenocorticotropic hormone (ACTH), 48 and 24 hours before killing, to ensure a consistent Na^+ uptake via maximal stimulation of mineralocorticotropic release from the adrenal glands (Hanke & Kloas, 1996). Frogs were hypothermally anesthetized and killed by decapitation. Lungs were removed and incised along the large pulmonary artery. Tissues were then dissected to flat sheets glued with the pleural side to Lucite rings and mounted in adapted Ussing chambers (effective aperture was 0.5 cm^2). Tissue preparations and mounting procedures were done as described in more detail by Fischer, Van Driessche and Clauss (1989). Both, apical and basolateral compartments, were continuously perfused with normal Ringer's solution (NRS) with a flow rate of 5 ml min^{-1}. All experiments were performed at 20–23°C.

Solutions and Chemicals

Normal Ringer's solution (NRS) was applied to both compartments of the Ussing chamber, containing in mM: 100 NaCl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES and 10 glucose (pH 7.4, adjusted with trizma base; all compounds were obtained from Fluka). In Ringer's solutions containing different Na^+ concentrations on the apical side (1–100 mM), the ion was replaced by equimolar amounts of NMDG (*n*-methyl-*d*-glucamine; Sigma). In experiments with apical Na^+ concentrations exceeding 100 mM, the osmolarity of the apical and basolateral solutions was adjusted with mannitol. Apical Ringer's solution was gassed with 20% O_2 at all times. To reveal the amiloride-sensitive component of the Na^+ current (I_{ami}), amiloride (10 μM; Sigma) was applied on the apical side. Gadolinium (100 μM) and BIG (benz-imidazolyl-guanidin, 0.3 mM) were added from stock solution to the apical solutions. PHMP (*p*-hydroxy-mercuribenzoic acid, 1 mM) was directly dissolved in apical solutions. $GdCl_3$, BIG and PHMB were purchased from Sigma.

Ussing-chamber Measurements

Modified 200-μl pipette tips were used for 1 M KCl/agar bridges to connect the bathing solution with the conducting electrodes (Ag/AgCl wires in 1 M KCl). The spontaneous transepithelial potential (V_t) was first determined and then clamped to 0 mV. The short-circuit current (I_{sc}) was recorded continuously on a strip-chart recorder (Kipp and Zonen, Netherlands). Additionally, data were digitized via an A/D transducer (MacLab, ADInstruments, Australia) and stored on computer (Apple LC II, USA). Transepithelial resistance was estimated according to Ohm's Law, from deflections of I_{sc} due to 2-mV voltage pulses of 2 seconds duration.

Single-channel Measurements

The α, β and γ subunit (2 ng cRNA each) of *Xenopus* ENaC (xENaC) from A6 nephron cell line were functionally expressed in *Xenopus* oocytes. Single-channel currents were recorded in the cell-attached configuration from devitellinized oocytes after 1 or 2 days of expression. The bath solution contained in mM: 90 KC1, 5 NaCl, 1 $CaCl_2$, 5 HEPES (pH 7.4). The pipette solution contained 100 NaCl, 1 $CaCl_2$, 5 HEPES (pH 7.4), with or without 100 μM Gd^{3+}. Currents were filtered at 100 Hz, amplified with a List LM-PC amplifier (List Electronics, Germany) and digitized by an Axon interface (1200 series; Axon Instruments, Foster City, CA). Data were acquired (2 kHz) and analyzed with the Axon pClamp software. Slope conductances were calculated from −100 to −40 mV. I/V relationships were fitted according to the Goldman-Hodgkin-Katz equation for currents (*see* Hillyard, Rios & Larsen, 2002). As a measure for channel activity, the product NP_o from recordings at −60 mV and at least 2 minutes duration was used.

Statistical Analyses

All values are presented as means ± standard error of the mean (SEM). Paired or independent Student's *t*-tests were used to estimate the significance between means. Significantly different values ($P < 0.05$) are labelled with asterisks (*), *n* marks the number of experiments and *N*, the number of oocyte donors.

Results

Electrophysiological Parameters of *Xenopus* Lung

Dissected tissues were mounted in a modified Ussing chamber and super-fused by symmetrical Ringer's solution. After a short time period of equilibration (3–5 min), transepithelial voltage (V_t) was measured (-5.8 ± 0.5 mV, $n = 50$) and clamped to 0 mV. Short-circuit current (I_{sc}) was then recorded continuously. Initial I_{sc} was 20.3 ± 1.45 μAcm^{-2}, and during 30 to 120 minutes the current equilibrated at 24.2 ± 1.3 μAcm^{-2}. Transepithelial resistance (R_t) was calculated at 313 ± 15 Ωcm^2 according to Ohm's law. Approximately 80% of the measured I_{sc} was inhibited by 10 μM apical amiloride (K_i 1.23 μM, Fischer et al., 1989). I_{ami} was determined at 19.6 ± 1 μAcm^{-2}.

Lung epithelia from control animals (no pretreatment with ACTH) exhibited decreased electrophysiological variables (V_t: -3.3 ± 0.4 mV, I_{sc}: 10.4 ± 0.7 and 11.7 ± 1 μAcm^{-2} after equilibration, I_{ami}: 8.16 ± 1 μAcm^{-2}, $n = 17$) but a similar R_t: (325 ± 21 Ωcm^2 $n = 17$).

Effect of Gadolinium on Electrophysiological Parameters

Apical application of 100 μM gadolinium resulted in a fast transient but significant increase of I_{sc} from 20.5 ± 2.9 to a peak of 35.7 ± 4.8 μAcm^{-2} and equilibrated within 20–30 minutes at a steady state of 28.3 ± 3.8 μAcm^{-2} ($n = 7$, Fig. 1A, Table 1). R_t was not affected significantly by the application of Gd^{3+} (Table 1). Gd^{3+} increased I_{sc} in a concentration-dependent manner, with an EC_{50} value of approximately 23.5 μM (Fig. 1*C*). The effect of Gd^{3+} on I_{sc} was fully sensitive to amiloride, no matter whether amiloride was added at maximal stimulation of I_{sc} or after its equilibration. As shown in Fig. 1*B* the lanthanide failed to stimulate I_{sc} in the presence of amiloride (control: 6.7 ± 0.9 μAcm^{-2}, Gd^{3+}: 6.7 ± 0.9 μAcm^{-2}, $n = 5$). Under Cl^--free conditions (Cl^- was substituted by SO_4^{2-}) the stimulating effect was still present, but basolateral addition of Gd^{3+} did not affect I_{sc} (*data not shown*).

Interaction of Gd^{3+} with the Na^+ Feedback-/Self-inhibition System in *Xenopus* Lung

Starting with 1 mM sodium in the apical bath, a stepwise increase of $[Na^+]_a$ up to 100 mM upregulated I_{sc}. A superfusion of the apical side with 200 mM Na^+, which was osmotically compensated by addition of 100 mM NMDG to the basolateral side immediately downregulated I_{sc} (Fig. 2*A*). The reduction of I_{sc} in these experiments must be attributed largely to the higher osmolarity of the superfused solutions. Therefore, in further experiments with high

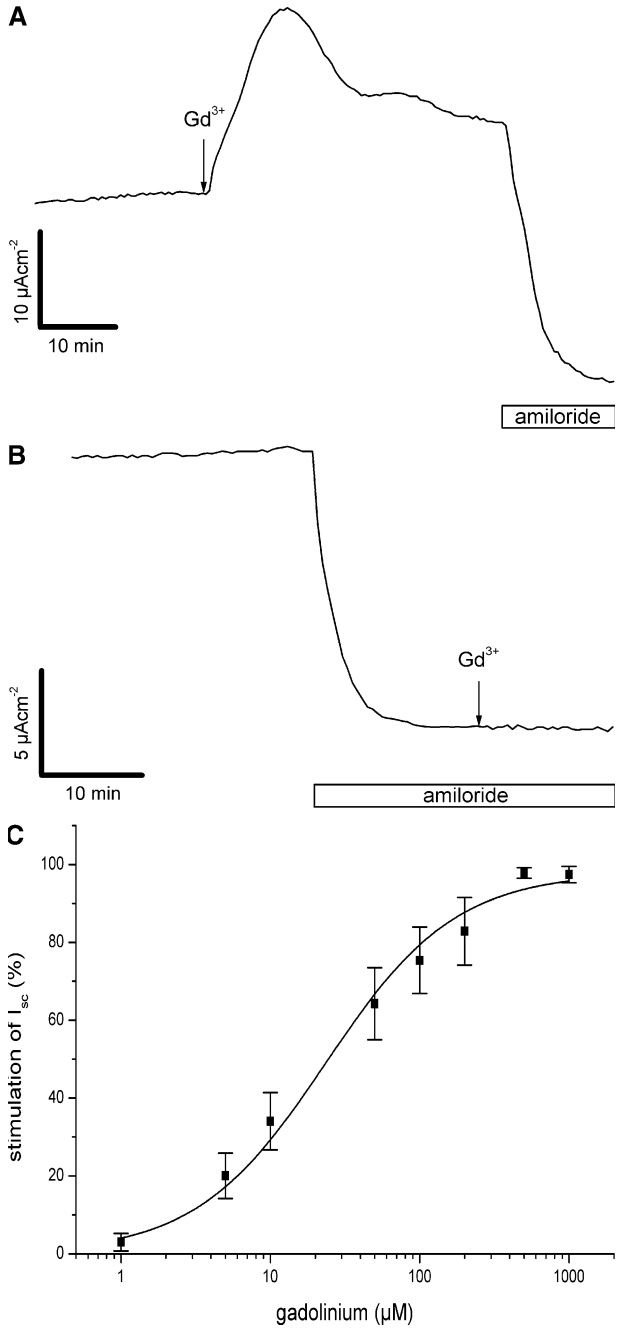

Fig. 1. Effects of apical application of Gd^{3+} on I_{sc} across *Xenopus* lung epithelia. (*A*) Original current trace from a typical experiment. Apical addition of 100 μM Gd^{3+} immediately increased I_{sc}, followed by equilibrium on an elevated level. (*B*) In the presence of amiloride (10 μM), gadolinium failed to affect I_{sc}. (*C*) Concentration-dependent effect of apical Gd^{3+} (0.001–1 mM) on I_{sc} across frog lung ($n = 10$). Maximal current was defined as 100% stimulation; values were taken at the peak of the effect. Half-maximal stimulation (EC_{50}) was determined with 23.5 μM Gd^{3+}. Data were fitted according to the Hill equation (Hill coefficient was 1).

Table 1. Effect of gadolinium (100 μM) on electrophysiological parameters

Parameters	Control	Gd^{3+}		Stimulation % (plateau)
		Peak	Plateau	
I_{sc}(μAcm^{-2})	20.46 ± 2.86	35.66 ± 4.89*	26.56 ± 3.64*	30.2 ± 3.5*
I_{ami}(μAcm^{-2})	17.15 ± 1.89	*n.d.*	23.56 ± 3.64*	36.1 ± 4.9*
R_t(Ωcm^{-2})	307 ± 26	322 ± 25	332 ± 27	*n.d.*
n	7			

Values of maximal stimulation: peak, values after equilibration: plateau. I_{sc}: short circuit current, I_{ami}: amiloride-sensitive current, R_t: transepithelial resistance, *n.d.*: not done.
Data are given as means ± SEM. *Values significantly different, with respect to control; $P < 0.005$.

Na^+ concentrations (120–200 mM), all solutions were adjusted to identical osmolarity. Under these conditions, I_{sc} decreased in a Na^+-dependent manner (Fig. 2*B*). If Gd^{3+} (100 μM) was tested under these high-Na^+ conditions (NaCl 200 mM) it increased the downregulated I_{sc} and this current was sensitive to amiloride (Fig. 2*C*).

BIG (benz-imidazolyl-guanidin)

Benz-imidazolyl-guanidin (BIG) was reported to increase amiloride-sensitive sodium absorption in various epithelia by releasing the Na^+ channels from Na^+-dependent inhibition (Friis & Nielsen, 2001; Turnheim, 1991). After application of 0.3 mM BIG (concentrations up to 1 mM were tested) on the apical side, we observed a slight but significant decrease of I_{sc} from 28.9 ± 4.2 to 27.2 ± 4.1 μAcm^{-2} ($P < 0.05$, $n = 6$). Interestingly, the presence of BIG prevented the stimulating effect of Gd^{3+} on I_{sc} and a supplementary application of Gd^{3+} further decreased I_{sc} to 25.6 ± 3.7 μAcm^{-2} ($n = 6$, Fig. 3). Although there was a considerable variability between the individual preparations, these effects proved to be significant in the paired Student's *t*-test ($P < 0.05$).

In further experiments, BIG was removed from the apical compartment (washout with NRS for at least 45 minutes) and epithelia were reexposed to apical Gd^{3+} for a second time. This procedure partially restored the sensitivity of I_{sc} to Gd^{3+}, but the effects were much weaker compared to control; I_{sc} then maximally rose about 3.4 ± 0.5 μAcm^{-2} ($P < 0.05$, $n = 5$).

PHMB (p-hydroxy-mercuribenzoic acid)

Experiments were performed with p-hydroxy-mercuribenzoic acid (PHMB), a compound that is believed to release negative feedback inhibition of ENaCs (Turnheim, 1991; Gilbertson & Zhang, 1998). PHMB was used in a concentration of 1 mM at the apical side of the tissues. In contrast to BIG, PHMB mimicked the effect of Gd^{3+} on I_{sc}. The maximal stimulation appeared within 10 minutes (from 23.7 ± 1.9 μAcm^{-2} up to 32.1 ± 2.5 μAcm^{-2}) and after 20–30 minutes, the current stabilized at a higher level (at 29 ± 2.6 μAcm^{-2}, $n = 8$). With PHMB in the solution (after equilibration), the addition of Gd^{3+} produced no significant effect on I_{sc} (Fig. 4 and Table 2). The current induced by PHMB was sensitive to amiloride. In the presence of amiloride, PHMB had no effect on I_{sc} (change from 3.0 ± 1.1 to 3.2 ± 1.3 μAcm^{-2}, $n = 5$). Reversed application of the compounds, with PHMB following the Gd^{3+}, confirms these results. PHMB did not further affect the current after I_{sc} had stabilized with Gd^{3+} in the solution (Table 2).

Effect of Gd^{3+} on Single-channel Currents

To investigate whether extracellular Gd^{3+} modulates the activity of the epithelial Na^+ channels in the cell membrane we performed patch-clamp experiments on *Xenopus* oocytes that express xENaC. In the cell-attached mode we found the open probability, given as NP_o, increased by Gd^{3+} (100 μM) in the pipette solution (NP_o: 0.72 ± 0.19 control, ($n = 9$, $N = 4$) and 1.79 ± 0.39 with Gd^{3+}, ($n = 9$, $N = 3$), (Fig. 5*A*, *B*). The single-channel amplitudes obtained at voltages from −100 to −20 mV were smaller when the patches were exposed to extracellular Gd^{3+} (Fig. 5*C*). In consequence, the slope conductances taken from −100 to −40 mV decreased from 5.5 pS (control) to 4.6 pS (Gd^{3+}).

Discussion

Na^+-Dependent Regulation of Epithelial Na^+ Channels

In the present study, dissected lungs from *Xenopus laevis* were used for Ussing-chamber experiments. These epithelia actively absorb Na^+, with Cl^- following passively—properties known from the adult mammalian lung (Fischer et al., 1989; Kim, 1990). With identical Ringer's solutions on both sides of the tissue, *Xenopus* lung exhibited a transepithelial Na^+ absorption and approximately 80% of I_{sc} was inhib-

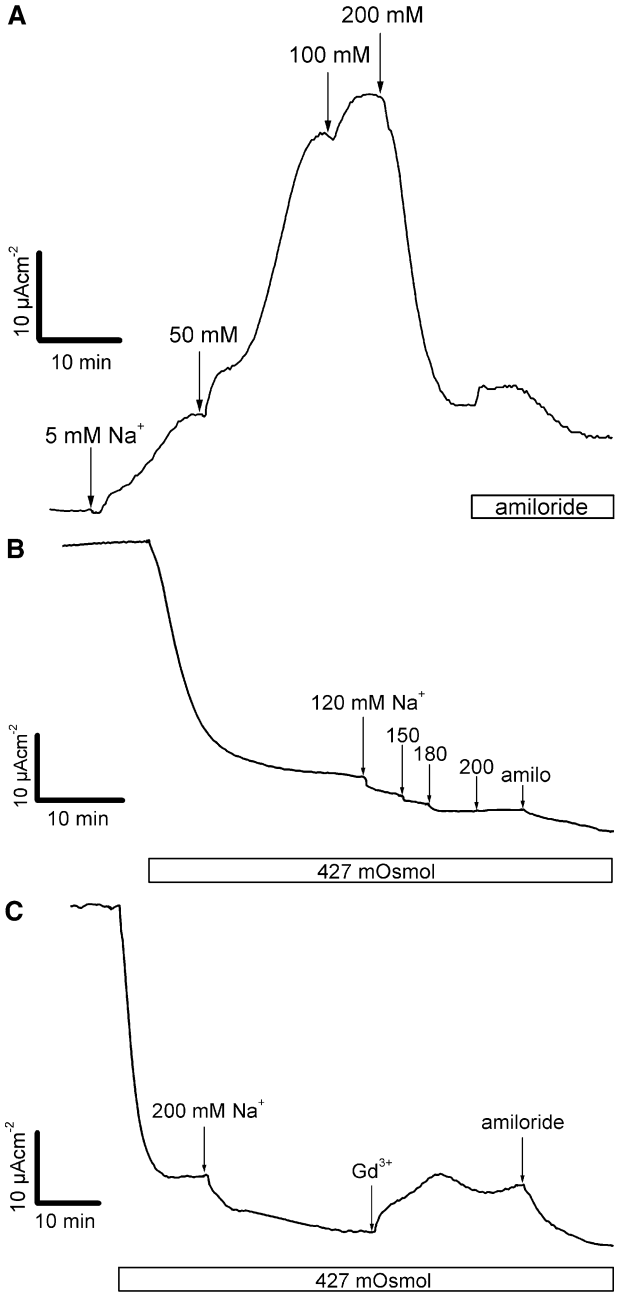

Fig. 2. Na^+-dependent effects on I_{sc}. (*A*) I_{sc} increased with changes in apical Na^+ concentration from 1 to 100 mM. Application of 200 mM Na^+ to the apical bath was accompanied by an addition of 100 mM NMDG to the basolateral solution to avoid osmotic gradients across the epithelium. Downregulation of I_{sc} was largely due to the hyperosmolar challenge by these solutions. The short transient increase of I_{sc} under amiloride (10 µM) was unique among 5 experiments. (*B*) Application of increasing Na^+ concentrations under constant osmolar conditions. After changing to high-osmolar solutions in both compartments (427 mOsmol) and equilibration of I_{sc}, the apical sodium concentration was increased in steps up to 200 mM. Finally, amiloride-sensitive current was determined (10 µM amiloride). (*C*) Similar experiment as shown in (*B*). After downregulation of I_{sc} in response to hyperosmolar conditions, 200 mM Na^+ was added on the apical side, followed by application of 100 µM Gd^{3+}. Note that the increase of I_{sc} in response to Gd^{3+} was fully sensitive to amiloride (10 µM).

ited by amiloride. The transepithelial resistance of the *Xenopus* lung was relatively low, but the electrophysiological variables for mammalian systems are mostly derived from cultured cells that may be affected by the conditions of cultivation (Jain et al., 2001). We found the earth metal Gd^{3+} to stimulate the Na^+ uptake and single-channel experiments revealed that Gd^{3+} modulates the epithelial sodium channels.

The Na^+ self-inhibition and the feedback inhibition, which may avoid excessive changes of intracellular Na^+ concentrations (Turnheim, 1991) and limit Na^+ absorption during periods of high salt delivery (Palmer, Sackin & Frindt, 1998) are known from various tissues (Turnheim, 1991). These mechanisms also control cloned epithelial Na^+ channels in the *Xenopus* oocytes (Puoti et al., 1997; Abriel & Horisberger, 1999) or, e.g., in transfected MDCK cells (Ishikawa, Marunaka & Rotin, 1998). Obviously, such Na^+-dependent short-term regulation appears in the alveolar epithelium from the *Xenopus* lung, too (Fig. 2*A*, *B*). From the present study we cannot answer whether this amphibian lung exhibits Na^+ feedback-inhibition, which is mediated by, e.g., the ubiquitin ligase Nedd4 (Staub et al., 2000; Harvey et al., 2001), or by as yet unidentified intracellular G protein-coupled factors (Komwatana et al., 1996; Dinudom et al., 1998; Abriel & Horisberger, 1999). Nevertheless, our results with compounds that interfere with self-inhibition suggest at least the presence of this control mechanism in *Xenopus* lung.

Effect of Gd^{3+} on Sodium Absorption

The earth metal gadolinium is known as an inhibitor of stretch-activated ion channels (SACs) (Caldwell et al., 1998; Reifarth et al., 1999) and acid-sensing ion channels (ASICs) (Babinski et al., 2000; Alien & Attwell, 2002). Further, Brown et al. (1993) reported a Ca^{2+}-sensing receptor that recognizes Gd^{3+} as an agonist. In fact, little is known about Gd^{3+} as an activator of ion channels. Addition of Gd^{3+} to the apical surface of the *Xenopus* lung induced a fast but transient increase of I_{sc}, followed by a recline to a steady but elevated level, without affecting R_t significantly (Table 1). The additional current was sensitive to amiloride and in the presence of this blocker, Gd^{3+} failed to stimulate I_{sc} (Fig. 1*A*, *B*). Friis and Nielsen (2001) reported a stimulation of transepithelial Na^+ transport across the frog skin, which was induced by Gd^{3+}, and attributed this effect to an interference of Gd^{3+} with the Na^+ self-inhibition and/or Na^+ feedback inhibition of epithelial Na^+ channels. Remarkably, in *Xenopus* lung Gd^{3+} stimulated the downregulated I_{sc} under high-Na^+ conditions and we determined a similar EC_{50} value for Gd^{3+} (23.5 µM) as Friis and Nielsen (2001) for the frog skin (23 µM).

Table 2. Effect of BIG or PHMB on Gd^{3+}-induced stimulation of transepithelial currents

Parameter	Control	BIG	+ Gd^{3+}
I_{sc}(μAcm^{-2})	28.93 ± 4.23	27.15 ± 4.05$^{\#}$	25.59 ± 3.79$^{\#}$
I_{ami}(μAcm^{-2})	26.22 ± 2.92	24.44 ± 2.69$^{\#}$	22.88 ± 2.45$^{\#}$
n		6	

	Control	PHMB		+ Gd^{3+}
		Peak	Plateau	
I_{sc}(μAcm^{-2})	23.7 ± 1.94	32.09 ± 2.52*	29.01 ± 2.75*	29.22 ± 2.49
I_{ami}(μAcm^{-2})	18.86 ± 2.22	n.d.	25.85 ± 2.28*	25.1 ± 2.1
n		10		

	Control	GD^{3+}		+ PHMB
		Peak	Plateau	
I_{sc}(μAcm^{-2})	28.26 ± 3.03	37.02 ± 2.76*	33.7 ± 3.76*	33.31 ± 3.91
I_{ami}(μAcm^{-2})	23.57 ± 1.26	n.d.	29.01 ± 1.99*	28.62 ± 2.06
n		5		

An additional application of compounds is indicated with +. BIG: benz-imidazolyl-guanidin (0.3 mM), PHMB: p-hydroxy-mercuribenzoic acid (1 mM).
Data are given as means ± SEM. Values significantly different from control: $^{\#}$, $P < 0.05$, *, $P < 0.005$; *n.d.* not done.

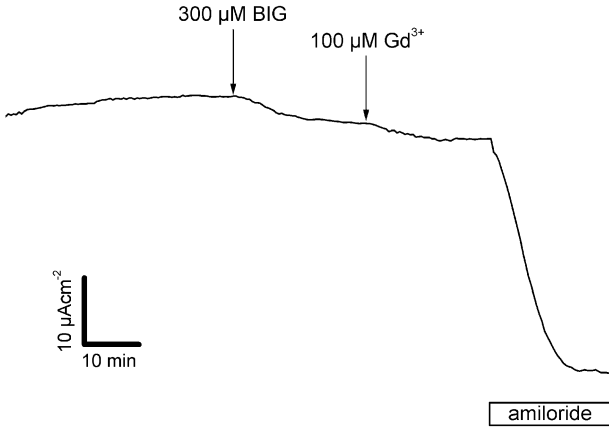

Fig. 3. Effect of apical application of BIG on I_{sc} across *Xenopus* lung epithelium. Shown is a current recording of a representative experiment. BIG (0.3 mM) slightly decreased I_{sc} and the addition of Gd^{3+} (100 μM) failed to increase the I_{sc}.

Benzimidazolyl-guanidine (BIG)

Benzimidazolyl-guanidine (BIG), as other related guanidine compounds, is reported to interfere with self-inhibition of Na^+ channels (Li & Lindemann, 1983; Lindemann, 1984; Turnheim, 1991; Palmer et al., 1998; Friis et al., 2001). The stimulating effect of BIG occurs via an increased number of open channels (Turnheim, 1991). In our experiments, application of BIG slightly decreased I_{sc} (Fig. 3), an obviously tissue-specific inhibition, as reported from prior studies (Li & Lindemann, 1983; Komwatana et al., 1998; Chraibi & Horisberger, 2002). Nevertheless, BIG not only prevented the stimulating effect of Gd^{3+} in *Xenopus* lung but induced a further decrease of I_{sc} when Gd^{3+} was added. In frog skin BIG abolished self-inhibition and activated transepithelial Na^+ transport to such a degree that Gd^{3+} failed to increase these currents any further (Friis & Nielsen, 2001). Friis and Nielsen suggested that both, BIG and Gd^{3+}, act on the same mechanism but bind to different sites (interaction with regulatory factors, i. e., the channel subunits themselves). Interestingly, in *Xenopus* lung, BIG did not increase I_{sc} by releasing Na^+ channels from Na^+-dependent autoregulating mechanisms but abolished the stimulation of Gd^{3+}. Our data support the suggestion that BIG and Gd^{3+} bind to different sites since Gd^{3+} further reduced I_{sc} in the presence of BIG. As postulated by other authors, a dual action of BIG is feasible. Either blocking Na^+ channels like amiloride (Li & Lindemann, 1983), and/or releasing them from self-inhibition through binding to another site. However, the mechanisms of these effects are still unclear.

P-Hydroxy-Mercuribenzoic acid (PHMB)

P-Hydroxy-mercuribenzoic acid (PHMB) belongs to sulfhydryl-reactive organic mercurials, which are known to inhibit Na^+-dependent intrinsic channel regulation (Lindemann, 1984; Turnheim, 1991). This compound increases amiloride-sensitive sodium currents in epithelial cells and this is contributed to conformational changes of the Na^+ channels (Turnheim, 1991). PHMB builds disulfide bridges with cysteines in extracellular portions from ENaCs

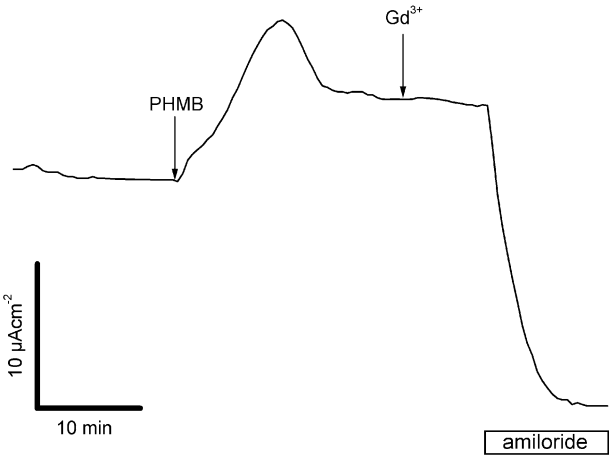

Fig. 4. Effect of PHMB on I_{sc} across *Xenopus* lung epithelium. 1 mM PHMB was added to the apical solution. Note: Subsequent application of Gd^{3+} (100 µM) after I_{sc} equilibrated in the presence of PHMB had no further effect on I_{sc}.

(Gilbertson & Zhang, 1998) and thereby increases channel activity. In *Xenopus* lung, PHMB increased I_{sc} and this effect was sensitive to amiloride. Remarkably, the effect induced by PHMB displayed the same characteristics as the upregulation of I_{sc} observed with Gd^{3+} (Fig. 4). The current rose to a peak value and reclined to an elevated steady-state level. After pre-stimulation with PHMB, Gd^{3+} had no further effect on I_{sc} (Fig. 4). Reversed application, with PHMB following Gd^{3+}, confirmed the lack of additive effects (Table 2). These observations indicate that Gd^{3+} and PHMB affected the same Na^+ channels, but not necessarily by interaction with the identical binding sites. The sulfhydryl-reactive PHMB fully mimicked the effect of Gd^{3+}, which indirectly suggests that the lanthanide also bound an extracellular site at the cell surface. An additional insertion of new channels into the apical membranes appears rather unlikely.

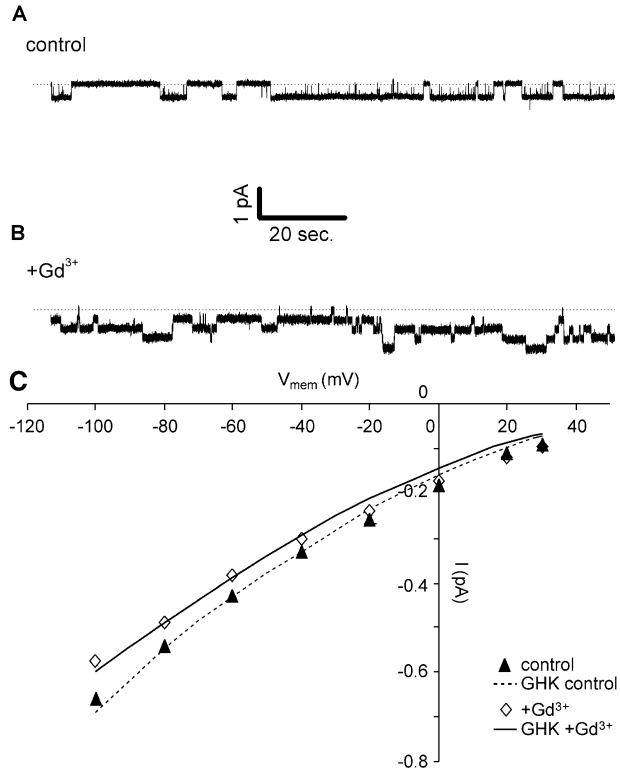

Fig. 5. Single-channel recordings from xENaCs expressed in *Xenopus* oocytes. Traces were recorded at −60 mV in the cell-attached configuration with Na^+ (100 mM) as conducting ion in the pipette. (*A*) Recording from control experiment without Gd^{3+} in the pipette solution. (*B*) Typical recording from experiment with 100 µM Gd^{3+} on the external side of the membrane. Gd^{3+} obviously increased channel activity. (*C*) Current-voltage relations (clamp protocol from −100 mV to +30 mV in steps of 10 mV) from cell-attached patches. Amplitudes are means of at least 10 measurements (N = 3–5). Slope conductance, calculated from −100 to −40 mV, was decreased from 5.5 (*control*) to 4.6 pS ($+Gd^{3+}$). Data were fitted according to the Goldman-Hodgkin-Katz (GHK) equation for currents. GHK-fits for external $[Na^+]$ = 100 mM, and intracellular $[Na^+]$ = 5 mM are indicated as lines. Calculated permeability (P_{Na}) was 1.74×10^{-12} (*control*) and 1.56×10^{-12} cm^{-3} s^{-1} ($+Gd^{3+}$) under these conditions.

Effect of Gd^{3+} on Single-channel Properties

The so-called DEG site in the vestibule of the outer pore region from DEG/ENaC (Kellenberger & Schild, 2002) plays a crucial role in channel gating and is possibly involved in Na^+ self-inhibition. Wild-type ENaCs cloned from rat (Kellenberger et al., 2002) or human (Snyder et al., 2000) proved to be insensitive to sulfhydryl-reactive compounds, but Gilberson & Zhang (1998) reported that PHMB released ENaC from Na^+ self-inhibition in rat taste receptor cells. This finding of PHMB uncoupling wild-type ENaCs from Na^+-dependent control may support speculations about an interference of PHMB or Gd^{3+} with this autoregulative mechanism. Patch-clamp studies demonstrated that sulfhydryl-reactive reagents can modulate gating in ENaCs with additional cysteines in the DEG site (introduced by site-directed mutagenesis). This was associated with high open probabilities due to long open dwell times and in some cases with reduced conductances (Snyder et al., 2000; Kellenberger et al., 2002). Interestingly, in cell-attached patches we found the open probability (NP_o) of the wild-type xENaC to be increased by external Gd^{3+} and, in patches with Gd^{3+} in the pipette, we found a reduction of conductance.

From these observations and our data, Gd^{3+} ions may directly interact with extracellular parts of the ENaCs. In this case the DEG site with its importance for channel gating and its putative role in Na^+ self-inhibition (Snyder et al., 2000; Kellenberger et al., 2002) or a further domain, the 'sodium receptor', as postulated by other authors (Puoti et al.,

1997; Palmer et al., 1998; Chraibi & Horisberger, 2002) could bind the trivalent cation.

In conclusion, we found that *Xenopus* alveolar epithelium exhibits a Na^+-dependent regulation of transepithelial Na^+ absorption. Apical gadolinium increased the amiloride-sensitive transcellular Na^+ transport under control conditions (100 mM NaCl) and under high-sodium conditions (200 mM NaCl), too. Application of BIG prevented the stimulating effect of Gd^{3+} on I_{sc}, whereas PHMB mimicked the effect of Gd^{3+}. Single-channel properties of xENaCs in *Xenopus* oocytes (open probability and conductance) were altered by extracellular Gd^{3+}. Gd^{3+} may directly interact with *Xenopus* epithelial sodium channels and thereby release them from Na^+-dependent self-inhibition. To elucidate the mechanism(s) by which Gd^{3+} can control Na^+ channels in detail awaits future investigations.

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