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Review

Annelid epithelia as models for electrogenic Na^+ transport

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Abstract

The electrogenic Na^+ absorption across tight epithelia from invertebrates follows the principles analog to the mechanisms found in vertebrates. Extracellular Na^+-ions pass the apical cell membranes through highly selective Na^+ channels and follow an electrochemical gradient which is sustained by the basolateral Na^+/K^+-ATPases. These apical Na^+ channels are selectively blocked by amiloride and represent the rate-limiting target for the control of transcellular Na^+ uptake. Although annelids express ADH-like peptide hormones, they lack the osmoregulatory mineralocorticoid system with the vertebrate-specific key hormone aldosterone. Thus, their epithelia may represent interesting models for investigation of ion transport regulation. While the formation of urine in the nephridia of, for example, leeches had been subject to intensive studies, the investigation of ion transport across their body wall was largely neglected. We use dissected segments of integuments from the limnic leech *Hirudo medicinalis* and, recently, from the earthworm *Lumbricus terrestris* for Ussing chamber experiments. We investigate transintegumental ion transport with focus on control of electrogenic Na^+ uptake and the amiloride-sensitive part of it and identified several extracellular factors as peptide hormones, tri- and divalent cations or purinergic molecules with regulatory effects on it. Meanwhile, there exists a macroscopic view on Na^+ absorption; however, other ion transport mechanisms across annelid integuments still await scientific effort. Here we present a concise synopsis about the electrophysiology of annelid integuments to illustrate the state of science and to evaluate whether further studies in this particular field may be of interest.

Keywords: *Hirudo medicinalis*; *Lumbricus terrestris*; Annelid integument; Sodium transport; Amiloride

1. Introduction

Various animals conquered osmotically unfavourable fresh water biotops by their ability to maintain a homeostatic internal milieu. Because body fluids of fresh water organisms are hyperosmotic to their environment, they developed mechanisms to compensate the loss of ions and the net-gain in water, therefore, they are able to absorb ions against large chemical gradients. The general model mechanisms of ion absorption and ion secretion were first postulated and described by pioneering work of physiologists as Krogh, Ussing and Zerahn.

Over the past decades, comprehensive work has been dedicated to investigation of salt homeostasis and transepithelial ion transports in limnic animals. Within time, frog skin emerged as the classical model for transepithelial ion exchange with a fresh water environment. Ussing and Zerahn showed that the net-Na^+ flux from apical to basolateral side resulted from an active process and was larger than calculated from electrochemical Na^+ gradient [1]. In 1958, Koefoed-Johnsen and Ussing [2] developed the two-barrier model of transcellular ion transport with Na^+ entering cells from the apical side by diffusion and then leaving by basolateral Na^+/K^+-ATPases. The intracellular Na^+ concentration was kept low while the K^+ level was high. K^+ leaves these cells passively through basolateral K^+-selective channels and does not contribute to the short-circuit current. The frog skin model gave direction to subsequent electrophysiological work on ion transporting tight epithelia to date.

Na^+ absorbing osmoregulatory epithelia are found in invertebrates in the gills, nephridia, intestine and the integument. An uptake of sodium or chloride across the integument of the fresh water leech *Aulacostomum sp.* was first documented in 1938 by Krogh [3]. Further reports followed from *Lumbricus terrestris* [4] and finally, amiloride-sensitive Na^+ conductances, a characteristic feature of trans-

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cellular Na^+ uptake in vertebrate tight epithelia, were described for the integument of the horse leech *Haemopis marmoralis* [5]. In the leech, the function of the nephridia has been thoroughly investigated. An osmotic inflow of water is compensated by an increased production of urine of up to two orders of magnitude. Under high-salt conditions, the final urine is decreased down to 10% of normal values [6,7]. Using electrical measurements in vivo and Ussing chamber experiments, Prusch and Otter [8] revealed basic differences of electrophysiological characteristics between the leech *Haemopis grandis* and *L. terrestris*. The integuments of these annelids showed inverse polarity of their transepithelial potentials. While in the leech the transepithelial potential was inside positive, the earthworm proved to be inside negative. The authors concluded an electrogenic Na^+ uptake across the leech integument which resembles the situation in the frog skin. As indicated by the inverse transintegumental potential, a different situation with chloride absorption was expected for the earthworm [8]. Since then, the regulation of transintegumental ion transport in annelids has been subject to very few and sporadic studies [9]. Approximately 10 years ago, we began to use isolated preparations of the dorsal integument from *Hirudo medicinalis* for Ussing chamber experiments [10]. The aim of these studies was to establish a physiological model for further investigations of transepithelial ion transport in limnic invertebrates.

2. Materials and methods

H. medicinalis were kept at room temperature (18–22 °C) without feeding in an artificial pond water (in mmol l^{-1}: 1 NaCl, 0.05 KCl, 0.4 $CaCl_2$ and 0.2 $NaHCO_3$, pH 7.4) or acclimated to high-salinity conditions (in mmol l^{-1}: 200 NaCl, 1 KCl, 0.4 $CaCl_2$ and 0.2 $NaHCO_3$, pH 7.4). Before the conceivably simple dissection, animals were hypothermally torpidized. After ventral incision, the intestine was detached and muscular layers were carefully scraped off. The dorsal integument of the subclitellar region was fixed on a needle-spiked ring with an aperture of 0.5 cm^2. Edges of the tissue were sealed up with silicone grease and mounted into an Ussing-type chamber. During experiments, both compartments were continuously perfused. The basolateral Ringer's solution contained (in mmol l^{-1}): 115 NaCl, 4 KCl and 1.8 $CaCl_2$. In the apical solution, KCl was replaced with TMA–Cl. In Na^+-free solutions, NaCl was substituted by equimolar concentrations of TMA–Cl. Ca^{2+}-free solutions contained 0.5 mmol l^{-1} EDTA. All experiments were performed at room temperature. First, the initial transepithelial potential (V_T) was measured and then clamped to 0 mV. The short-circuit current (I_{sc}) was recorded continuously and the transepithelial resistance (R_T) was deduced from superimposed 20 mV pulses. The amiloride-sensitive current (I_{ami}) was measured by the decrease of I_{sc} upon apical presence of 100 μmol l^{-1} amiloride. I_{Na}, the transepithelial Na^+ transport, was determined by substitution of apical NaCl by equimolar TMA–Cl. Re-addition of apical Na^+ re-established the I_{sc}.

3. Results

3.1. Transepithelial Na^+ uptake

The integument of *H. medicinalis* proved to be a tight epithelium with an average transepithelial resistance R_T of about 2 kΩ cm^2 (Table 1a). The initial V_T was inside positive which indicated absorption of cations and secretion of anions. There is an electrochemical gradient for Na^+ which was generated by ouabain-sensitive basolateral Na^+/K^+-ATPases [10]. Part of the apical Na^+ conductances (approximately 45% of I_{Na}) were sensitive to amiloride with a K_i-value of approximately 2.9 μmol l^{-1} [11], a low affinity in comparison to amiloride-sensitive epithelial Na^+ channels from vertebrates. The non-Na^+ currents comprised approximately 60% of I_{sc} (Table 1a) and probably resulted from secretion of Cl^- ions.

High apical Na^+ concentrations as well as a rise in cytosolic Na^+ concentrations induce self-inhibitory or negative feedback regulation of apical Na^+ conductances of epithelial cells from vertebrates [12,13]. Current models of Na^+ feedback inhibition postulate an intracellular, as yet unidentified, Na^+-sensing receptor that mediates an ubiquitine-protein ligase-dependent endocytosis and degradation of apical Na^+ channels through G-protein-coupled mechanisms [14,15]. Therefore, the feedback inhibition is due to a reduction of total number of surface-expressed channels. From previous studies, we know that maximal activation of Na^+ transport across the leech integument is observed at around 20 mmol l^{-1} Na^+ in the apical solutions and increased amounts of Na^+ already reduce the Na^+ uptake due to these autoregulative processes (personal observations; Ref. [11]). Under apical low-Na^+ conditions, with a concentration as low as 2 mmol l^{-1}, transepithelial Na^+ uptake is tremendously upregulated and this additional

Table 1
Electrophysiological variables of *H. medicinalis* dorsal integuments

	(a) Pond water ($N=16$)	(b) High salinity ($N=16$)
V_T [mV]	32.7 ± 3.9	16.2 ± 2.5*
R_T [Ω cm^2]	2038 ± 346	1701 ± 176
I_{sc} [μA/cm^2]	18.96 ± 1.76	9.56 ± 1.1*
I_{ami} [μA/cm^2]	4.34 ± 0.68	3.12 ± 0.48
I_{Na} [μA/cm^2]	7.66 ± 0.82	4.6 ± 0.54*

Data are obtained in (a) pond water and (b) high-salinity acclimated animals. Compartments were perfused with 115 mM NaCl solutions. Values are means ± S.E.M., $N=16$.
V_T, transepithelial potential; R_T, transepithelial resistance; I_{sc}, short-circuit current; I_{ami}, amiloride-sensitive current; I_{Na}, transepithelial Na^+ current.
* Significantly different from pond water-values: $P<0.005$.

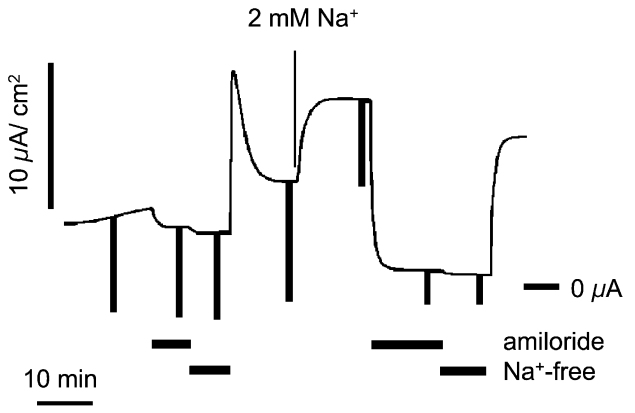

Fig. 1. Effect of apical Na^+ concentrations on I_{sc} across leech integument. After I_{sc} equilibrated with symmetrical solutions (115 mmol l^{-1} NaCl in both compartments), apical compartment was perfused with 2 mmol l^{-1} NaCl solution. Afterwards, the current sensitive to 100 µmol l^{-1} amiloride (I_{ami}) and total Na^+ transport were determined.

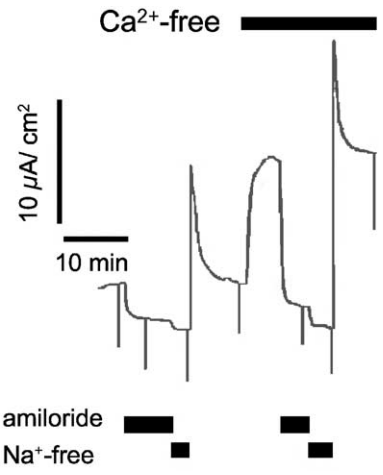

Fig. 2. Effect of apical Ca^{2+}-free conditions on I_{sc} across integument of *H. medicinalis*. Original recording of short-circuit current (I_{sc}) from leech integument. After Ca^{2+}-free Ringer's solution perfused the apical compartment, the amiloride-sensitive Na^+ current was immediately upregulated.

current is completely sensitive to amiloride (Fig. 1). These autoregulative mechanisms that correlate the magnitude of transepithelial Na^+ absorption to extracellular apical Na^+ concentrations represent short-term adaptive processes and have been reported for various tight epithelia [16].

Although effects of long-term exposure to high-salinity conditions on energy, ionic and volume regulation have been subject to previous studies, the ion conductances of the epithelial body walls have not been investigated. To investigate whether long-term acclimation processes affect the transintegumental ion conductances, we exposed leeches up to 10 days to high-saline conditions (200 mmol l^{-1} NaCl). The plasma Na^+ contents from leeches acclimated to fresh water are stated with ~ 115–140 mmol l^{-1} [8,17] and 200 mmol l^{-1} environmental Na^+ probably changes the physiological situation from absorption to an excretion of intruded excess Na^+-ions, a physiological challenge which the animals managed obviously. The initial V_T was reduced in comparison to pond water integuments (Table 1b). Although high-salt acclimation tended to result in reduction of total I_{Na}, the amiloride-sensitive transcellular Na^+ transport I_{ami} was not significantly changed (Table 1b). Thus, high-salt acclimation left the amiloride-sensitive transcellular Na^+ transport unaffected.

3.2. Regulation of Na^+ uptake by apical Ca^{2+} and Gd^{3+}

The leech integument exhibits a low permeability for Ca^{2+}-ions and the apical presence of micromolar concentrations of Ca^{2+} downregulates I_{sc} [11]. Na^+ autoregulatory processes elevate intracellular Ca^{2+} concentrations [18] which in turn inhibit the amiloride-sensitive Na^+ channels either directly or by protein kinase C-mediated mechanisms [19,20]. A total removal of Ca^{2+}-ions from the apical compartment enhanced I_{sc} markedly (Fig. 2) which is due to an upregulated I_{Na} of additional 119 ± 38% (N=5) but the percentage of the amiloride-sensitive Na^+ currents remained unaltered. Only the downregulated Na^+ transport in the presence of high apical Na^+ concentrations can be stimulated by a removal of apical Ca^{2+}-ions (personal observation; Ref. [11]). Whether apical Ca^{2+} interacts with apical Na^+ channels or membrane structures is not clear. Recently, extracellular Ca^{2+}-ions have been identified as first messengers that bind to G-protein-coupled receptors, the CaRs, which employ intracellular Ca^{2+}-signaling via phosphoinositide pathways [21,22]. The lanthanide gadolinium (Gd^{3+}) was reported to be an agonist for these CaRs [22,23]. In our experiments, apical application of Gd^{3+}-ions in concentrations <0.1 mmol l^{-1} weakly decreased I_{sc} across the leech integument (Fig. 3). Higher amounts of Gd^{3+} significantly upregulated I_{Na} in high-saline-acclimated leeches up to 538 ± 150% (N=5) with an EC_{50}-value of approximately 1 mmol l^{-1}, whereas in pond water integu-

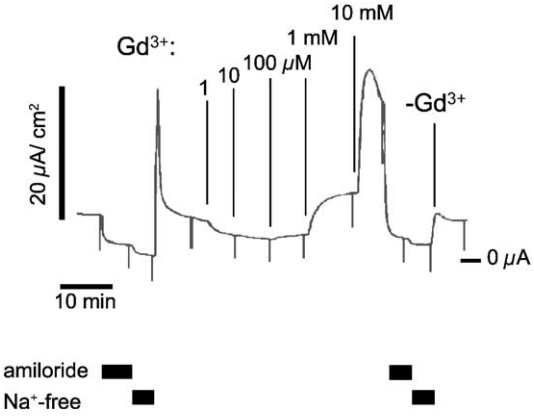

Fig. 3. Original recording of short-circuit current (I_{sc}) obtained from integument of *H. medicinalis* after acclimation to high saline conditions (200 mmol l^{-1} NaCl). Gd^{3+} was added in increasing concentrations to the apical compartment. Note the sensitivity of the Gd^{3+}-induced current to amiloride.

ments, there was a minute effect (105 ± 28%; $N=5$). The residual non-Na^+ currents were not affected (not shown). The stimulation of I_{Na} was reversible by wash-out of Gd^{3+} (Fig. 3) and was prevented in the presence of amiloride. A basolateral application of Gd^{3+} was without effect upon ion conductances and indicated apical one-sidedness of its action (data not shown).

3.3. Hormonal regulation

The apical Na^+ channels in the leech integument were largely stimulated by intracellular cAMP [10] and this upregulation of transepithelial Na^+ current was at least partly due to an increase in the total number of surface expressed Na^+ channels [10]. A stimulation of electrogenic Na^+ transport by intracellular cAMP was also observed in the leech caecal epithelium [24], a leaky epithelium with a V_T close to 0 mV and a R_T of 61 Ω cm^2 which lacks the amiloride-sensitive Na^+ channels [24].

Peptide hormones often mediate a short-term control of ion transports by binding to G-protein-coupled receptors and subsequent stimulation of adenylyl cyclases. Invertebrate hormones as lysine–conopressin or angiotensin II amide were isolated from neuronal tissue from the rhynchobdellid leech *Theromyzon tessulatum* or the pharyngobdellid *Erobdella octoculata* [25–28] and these peptides are obviously homologs either of the vertebrate antidiuretic hormones vasopressin and vasotocin or the angiotensin II, respectively. Because the natural factors for the increase in cytosolic levels of cAMP in vivo were not known, these peptides were tested for their effects on transepithelial ion conductances across the leech integument and found to be rather inhibiting to I_{sc} [9,29]. Lysine–conopressin induced a transient increase of I_{sc} but finally reduced Na^+ transport (Fig. 4). The vertebrate hormones 8-arginine–vasopressin (Arg–vasopressin) and 8-lysine–vasopressin (Lys–vasopressin; e.g. from pigs) downregulated the amiloride-sensitive Na^+ transport, an effect which seemed to mimick the

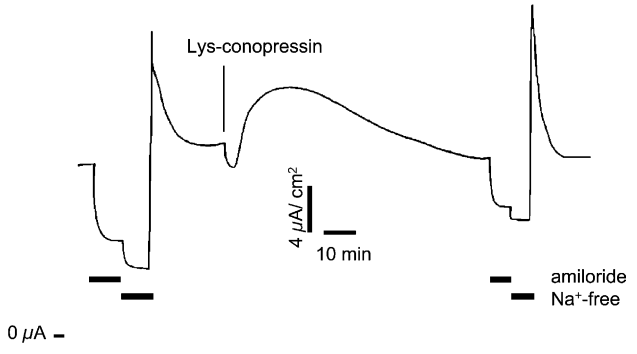

Fig. 4. Effect of Lys–conopressin on I_{sc} of integument of *H. medicinalis*. Original recording of short-circuit current (I_{sc}) from leech integument. Lys-conopressin (5 µmol l^{-1}) was added to the basolateral solution. The current sensitive to 100 µmol l^{-1} amiloride was determined before apical superfusion with Na^+-free Ringer's solution.

effects of the conopressin [29]. Lys–vasopressin induced in opposite to Arg–vasopressin, an immediate and pronounced downregulation of I_{ami} and I_{Na} [29]. In search of further neuronal factors, a novel peptide, the leech osmoregulator factor (LORF), has been identified. An application of the LORF (IPEPYVWDamide) [28] reduced I_{ami} and I_{Na} by approximately 30% and this osmoregulating effect reasoned the nomenclature. Another peptide from leeches, GDPFLRFamide [25], which was assumed to affect the transintegumental ion transport, increased Cl^- secretion but did not affect Na^+ uptake [29]. Therefore, the hormonal messenger for a cAMP-mediated positive onset of transintegumental Na^+ transport has not been detected yet. The neurotransmitter dopamine from which presence was verified for the leech central nervous system again induced a pronounced decrease of I_{sc} and I_{ami} [29,30].

3.4. Auto-/paracrine osmoregulatory mechanisms

The body surface of annelids is covered with a layer of mucus. Besides a variety of other functions, this slimy coat serves as mediator for extracellular messengers and for excretion of various matters. Mucopolysaccharides and ions, which are believed to provide a kind of microenvironment in direct proximity of the apical cell membranes, possibly affect transintegumental ion transport regulation. The mechanisms that mediate mucus secretion and that affect its composition have not been investigated for annelid integuments. In other integuments as frog skin, mucus secretion by exocytotic processes is correlated to cAMP-related mechanisms and a Cl^- secretion [31]. Flux studies verified net-Cl^- secretion through integuments of *H. grandis* [8], but whether similar processes drive the mucus production is not clear yet.

Apical surface expression of a variety of membrane receptors has been shown in various epithelia where they act among other tasks in the para- and/or autocrine control of transepithelial ion conductances. ATP, UTP or adenosine, for example, may be agonists locally released from epithelial cells [32] and a reciprocal regulation by these molecules with decreased Na^+ absorption and stimulation of Cl^- secretion is a common principle of ion transport control in vertebrate epithelia [33,34]. Nucleotides regulate ion transport via P2X- and P2Y-receptors [35] which may be strictly distributed to either the apical or to the basolateral membranes. In general, P2X- and P2Y-receptors induced control of Na^+ absorption or Cl^- secretion is mediated by an increase of intracellular Ca^{2+} [33,36]. We evaluated the regulatory impact of extracellular nucleotides upon ion transport regulation in the leech integument. Examination of pond water- or high-salt-adapted preparations (200 mmol l^{-1} NaCl) indicated different sensitivities to these agonists. The stimulatory effect of ATP on transcellular Na^+ uptake was exerted from both sides of the integument with an EC_{50}-value of approximately 80 µmol l^{-1} and appeared after high-saline adaptation while we were unable to detect

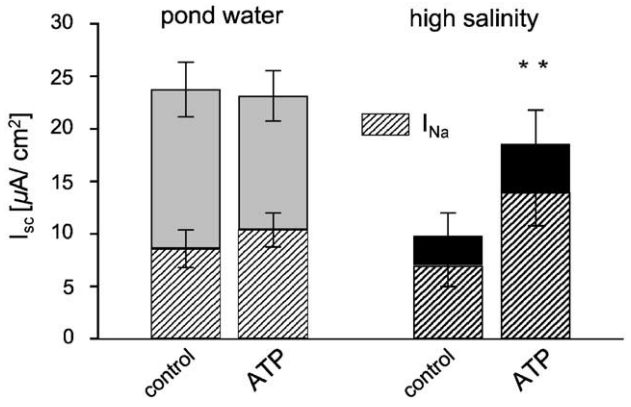

Fig. 5. Effect of basolateral application of ATP on short-circuit current (I_{sc}) of pond water- or high-salt-adapted integuments of *H. medicinalis*. Transepithelial Na^+ uptake (I_{Na}) was determined before (control) and after ATP (1 mmol l^{-1}) was added to the basolateral solution. Hatched bars, Na^+ current. Filled bars, residual non-Na currents. Values were significantly different from control: **: $P<0.005$ ($N=5$).

any significant sensitivity of I_{sc} to ATP in integuments from pond water (Fig. 5). UTP left the macroscopic transepithelial ion conductances of the leech integument unaffected (data not shown). A maximal stable and non-transient activation of I_{sc} by administration of adenosine was only observed in high-salt specimen with an EC_{50}-value of 0.17 mmol l^{-1}. The net-increase was due to a stimulation of non-Na^+ currents (Fig. 6). This action of adenosine was exclusively restricted to the basolateral side (data not shown).

3.5. Earthworm integument

Recently, we extended our investigations on the integument of the earthworm *L. terrestris*. These animals have to cope with similar problems as fresh water annelids, a permanent bias of losing electrolytes and a further challenge is represented by the danger of desiccation. In earthworms, the basolateral administration of ouabain uniformly shifts I_{sc} to more negative values which indicates active Na^+ absorption and with a high paracellular resistance of 2.46–24.5 MΩ cm^2, the paracellular passage of Na^+ is almost out of question. The net-Na^+ uptake is sensitive to amiloride but this effect underlies a large variability (S. Krumm, personal observations). In fact, this inconsistent response of the earthworm integument to amiloride seems to be an unusual case of seasonal adaptation of transcellular Na^+ transport which was described previously [37]. A comparison of electrophysiologic characteristics from earthworm with the data which we obtained from the leech integument revealed basic differences. In opposite to *H. medicinalis*, the earthworm exhibits an inside negative transintegumental potential (-10.8 ± 1.2 mV; $N=14$). This is accompanied by a higher R_T of up to 10 kΩ cm^2 (Fig. 7a). The transepithelial current starts with a negative value indicating a net-uptake of anions (Fig. 7b). Apical administration of the loop diuretic furosemide increased I_{sc} (S. Krumm, personal observations), however, since the furosemide-sensitive Na^+–K^+–$2Cl^-$ cotransporter is electroneutral, the presence of further apical Cl^- conductances remains to be demonstrated. In summary, the situation in the earthworm integument with a net-absorption of anions does not resemble the classic model of Na^+ transport as known from frog skin.

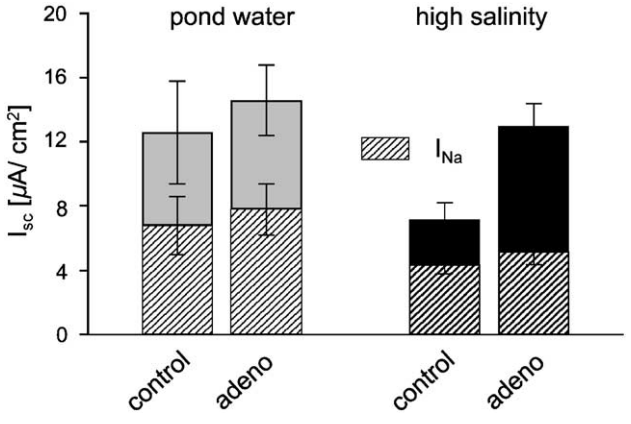

Fig. 6. Effect of basolateral application of adenosine upon short-circuit current I_{sc} of pond water- or high-salt-adapted integuments. Transepithelial Na^+ uptake was determined after I_{sc} reached steady state (control) and after 10 mmol l^{-1} adenosine was added to the basolateral solution (adeno). Note that I_{Na} (hatched columns) was not affected. Filled bars, residual non-Na currents. Values were significantly different from control: **: $P<0.005$ ($N=5$).

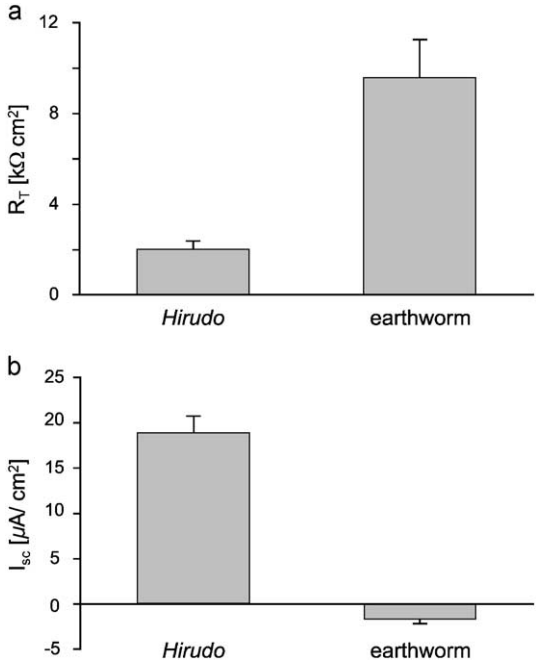

Fig. 7. Comparison of electrophysiological variables obtained from integuments of *H. medicinalis* or *L. terrestris*. (a) Transepithelial resistance R_T and (b) initial short-circuit current I_{sc} from earthworm ($N=14$) and leech integument ($N=16$).

4. Discussion

Many ion channels and transporters belong to phylogenetically old protein families with members found throughout the animal kingdom. Consolidated data concerning a molecular characterization of integumental ion channels or transporters from annelids and their regulation are no more than poor. The apical passage of Na^+-ions is the limiting step for transepithelial Na^+ uptake and the target site for its regulation [13]. The inhibition of transcellular Na^+ current by micromolecular concentrations of amiloride is specific for Na^+ absorbing tight epithelia [13]. Meanwhile, an overriding number of these highly amiloride-sensitive epithelial Na^+ channels, the ENaCs, were cloned from different vertebrate species [38,39]. The nematode *Caenorhabditis elegans* expresses proteins, the so-called degenerins, which were originally identified in neurodegenerative mutants and they belong with the ENaCs to one protein superfamily. The genome of *C. elegans* has been fully sequenced and, obviously, this animal lacks the characteristic ENaC-type cation channels known from vertebrate tight epithelia [40]. The leeches possess amiloride-sensitive epithelial Na^+ channels and therefore may contribute an evolutionary link between osmoregulation of primitive nematodes and higher organized animals as *Drosophila* from which two ENaC-type orthologues have already been cloned [41,42]. A relationship of these Na^+ channels from leeches with the well-characterized ENaCs and their degree of identity still remains to be shown.

In vertebrates, one important hormonal regulatory pathway for ion homeostasis is the mineralocorticoid system with the key hormone aldosterone. This steroid emerged in the evolution of teleosts and appears throughout the vertebrate phylum [43]. Besides certain short-term adaptive processes, it is known to mediate long-term adaptation on the gene expression level of epithelial ion transport proteins [44,45]. Invertebrates lack aldosterone and in fact they seem to have no equivalence. Thus, with respect to long-term osmoregulation, an investigation of the phylum of annelids may reveal details of properties from ancestral ion transport mechanisms and their regulation.

There is evidence that the neuroendocrine system of limnic invertebrates with ion transporting body walls as, for example, in the pulmonate freshwater snail *Lymnea stagnalis*, is involved in control of Na^+ homeostasis [46,47]. The synthesis of a sodium influx stimulating peptide (SISP) was increased when snails were exposed to hyposmotic medium and decreased when placed into hyperosmotic environment. Application of the SIS-peptide to preparations of head-skin from *L. stagnalis* increases V_T (inside positive) and I_{sc}. The stimulation is long-lasting and persists for longer than 30 min after wash-out [48]. In contrast to other peptide hormone-induced fast adaptive processes, this may represent a kind of long-term acclimation in invertebrates. Such peptide hormones of neuronal origin may control salt homeostasis as well in annelids and meanwhile expression of a SISP-like peptide was demonstrated for the brain of the freshwater leech *T. tessulatum* [49] where it plays an osmoregulatory role.

Since *H. medicinalis* live in fresh water and reach only exceptionally brackish water [50], they cannot be expected to be euryhaline organisms. Nevertheless, *H. medicinalis* exhibit an astounding ability of hyperosmotic acclimation. They have been shown to acclimate up to 16‰ salinity within days and behave as hyperosmotic osmoconformers [51] which survive there for almost a year [6,51]. During the initial phase, they passively lose water and take up salts. The Na^+, K^+ and Cl^- concentrations of the body fluids are largely increased and this is accompanied by an accumulation of these short-chain carboxylic acids. As in fresh water, the leeches maintain as well under hypersaline conditions a relatively low extracellular Cl^- concentration and one important anion fraction in body fluids comprises short-chain carboxylic acids that originate from glycolytic pathway and citric acid cycle. An extrusion of excess salt by the nephridia should then balance the total Na^+ uptake that the extracellular volume was restored within a few days [51]. We did not find the macroscopic electrophysiological variables, including I_{Na}, to be greatly affected by a long-term exposure to high salinity. Nevertheless, the acclimation enhanced the stimulating effect of Gd^{3+} from a doubling of I_{Na} in pond water-integuments to a more than six-fold increase of I_{Na} in high-salt integuments. Therefore, the latter condition may induce expression of more silent channels or Gd^{3+}-binding structures that trigger the activation of Na^+ conductances. Further, these adaptive processes conferred sensitivity of I_{Na} to stimulation by ATP or of non-Na^+ currents to adenosine in high-saline integuments.

The question of how apical Na^+ entry from very diluted pond water is energized was explained by the detection of proton-V-ATPases in the apical plasma membranes [52]. The frog skin constitutes one model for V-ATPase-dependent Na^+ transport [53] and meanwhile, such absorption mechanisms have been demonstrated for further fresh water-inhabiting species such as fish, amphibians and crustaceans [54]. However, whether H^+ secretion is used for driving apical Na^+ entry in integuments of annelids remains yet to be shown. Of course the Na^+ gradient can be expected to energize secondary active transport mechanisms as Na^+–HCO_3^--cotransport (NBC) or the Na^+–H^+-exchange (NHE). These transport systems are electroneutral and may be involved in the Na^+ absorption. The NBC is present in leech glial cells [55], but an expression in epithelia awaits to be proven. In crustaceans, an electrogenic member of the NHEs with a transport stoichiometry of $2Na^+/1H^+$ has been cloned. It is expressed in osmoregulatory gill epithelium and hepatopancreatic cells [56]. Interestingly, this electrogenic exchange is sensitive to amiloride and there is obviously no orthologue in vertebrates.

Transepithelial ion transport is influenced by external Ca^{2+}-ions and the regulatory impact of apical calcium is probably tissue-specific [43,57,58]. Besides an upregulation

of I_{sc} after total removal of apical Ca^{2+}, there have been studies that demonstrated upregulation by high extracellular Ca^{2+} concentrations [58]. Removal of apical Ca^{2+}-ions shuts down transcellular Ca^{2+} transport and in consequence reduces intracellular Ca^{2+} a substrate for basolateral Na^+/Ca^{2+} exchangers [59,60]. These electrogenic $3Na^+-1Ca^{2+}$ exchangers (NCE) play their critical role in processes that require Ca^{2+} extrusion from cells [59]. These antiporters have a high capacity of transport and following the electrochemical Na^+ gradient, they can produce Ca^{2+} fluxes into both directions. Taking the immediate and large stimulation of I_{sc} by apical removal of Ca^{2+}-ions into account, one can speculate about NCEs in the leech integument, but their presence has to be verified first. A large experimental field awaits further studies.

Further investigations revealed the presence of voltage-dependent Na^+ conductances in the dorsal integument of *H. medicinalis*. Their activation is rather slow and is inhibited by apical presence of divalent cations [61]. Their characteristics resembled voltage-dependent activation of Cl^- conductances which were reported for toad skin or fish intestinal epithelium [62,63]. Clarification of the physiological relevance and the natural situation of activation of these conductances has not been prosecuted any further. In comparison to the scope of data concerning Na^+ or cation transport across the leech skin, the investigation of transintegumental Cl^- transport appears rather neglected and the data are scarce [9]. After, under apical Na^+-free conditions, the electrogenic Na^+ transport, I_{Na}, has been tied up, there is a considerable residual non-Na^+ current (Table 1a) which may be attributed to an electrogenic secretion of Cl^- ions. This current is likely to be transcellular and is upregulated by cyclic AMP [11], but the molecular identity of these Cl^- conductances has not been clarified. In opposite to earthworm integument, there is no evidence for electroneutral $Na^+-K^+-2Cl^-$−cotransporter activity since loop diuretics as, for example, furosemide, have no effect (personal observation; Ref. [8]).

4.1. Perspectives

The investigation of transepithelial ion conductances with Ussing chambers has a long tradition but this approach allows a rather macroscopic electrophysiological characterization of ion transports. It is a valuable tool for obtaining a general view over electrogenic transepithelial processes and often represents the starting point for future more fine-tuned investigations.

The existing data from studies of transintegumental ion transports indicate the electrophysiological situation in the leech skin to resemble principles that were verified for the classic model of frog skin. These integuments represent intact multicellular epithelia with the complete assortment of different types of cells. Probably, there is a specialization of single cells, as known, for example, from mitochondria-rich cells in amphibian skin, and a division in their particular physiological tasks. We reported some regulatory effects on transintegumental ion transport, but the molecular characterization of involved membrane proteins is scarce and even the degree of relationship from the annelid amiloride-sensitive Na^+ channels with the ENaCs from vertebrates remains speculative. The nematode *C. elegans* expresses the degenerins but the ENaCs obviously emerged elsewhere in evolution, perhaps at the level of the molluscs and the articulata. The genome of the arthropod *Drosophila melanogaster* encodes at least 22 different members of the DEG/ENaC-protein superfamily [40] with only two ENaC-orthologues to be characterized. The presented short synopsis about their electrophysiology may indicate integuments of annelids as promising models for transepithelial ion transport regulation. To give further work on these invertebrate epithelia may be rewarded by surprises as, for example, the identification of the electrogenic crustacean $2Na^+/1H^+$ transporter.

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