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The Electrical Potential Difference through the Foot Epithelium of the Snail *Achatina achatina, Lameere* during Mechanical and Chemical Stimulation

Tomasz TYRAKOWSKI, Iga HOŁYŃSKA, Magdalena LAMPKA and Piotr KACZOROWSKI

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An important electrophysiological variable – the transepithelial potential difference reflects the electrogenic transepithelial ion currents, which are produced and modified by ion transport processes in polarized cells of epithelium. These processes result from coordinated function of transporters in apical and basolateral cell membranes and have been observed in all epithelial tissues studied so far. The experiments were performed on isolated specimens of snail foot. In the experiments, the baseline transepithelial electrical potential difference – PD, changes of transepithelial difference during mechanical stimulation – dPD and the transepithelial resistance were measured with an Ussing apparatus. A total of 60 samples of foot ventral surface of 28 snails were studied. The transepithelial electrical potential difference of isolated foot ranged from –6.0 to 10.0 mV under different experimental conditions. Mechanical stimulation of foot ventral surface caused changes of electrogenic ion transport, observed as transient hyperpolarization (electrical potential difference became more positive). When the transepithelial electrical potential difference decreased during stimulation, the reaction was described as depolarization. When amiloride and bumetanide were added to the stimulating fluid so that the sodium and chloride ion transport pathways were inhibited, prolonged depolarization occurred. Under the influence of different stimuli: mechanical (gentle rinsing), chemical (changes of ion concentrations) and pharmacological (application of ion inhibitors), transient changes of potential difference (dPD) were evoked, ranging from about –0.7 to almost 2.0 mV. Changes in transepithelial potential difference of the pedal surface of the snail's foot related to these physiological stimuli are probably involved in the locomotion of the animal and are under control of the part of the nervous system in which tachykinin related peptides (TRP) act as transmitters.

Key words: Electrophysiology, ion transport, integument, snail *Achatina achatina*.

Tomasz TYRAKOWSKI, Iga HOŁYŃSKA, Magdalena LAMPKA, Piotr KACZOROWSKI, Department of Pathobiochemistry and Clinical Chemistry, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland.
E-mail: tomtyr@cm.umk.pl

The epithelium, forming the external part of the gastropod integument, generates the transepithelial electrical potential over the whole animal body (ISTIN & FOSSAT 1972; ENYIKWOLA & BURTON 1983; SIMKISS 1988). This important electrophysiological variable directly reflects the electrogenic transepithelial ion currents, which are produced and modified by ion transport processes in polarized cells of epithelium. These processes result from coordinated function of transporters localized in apical and basolateral cell membranes (GREGER 1996; TYRAKOWSKI *et al*. 1997b; KOSIK-BOGACKA & TYRAKOWSKI 2001a).

The transepithelial electrical potential difference has been observed in all epithelial tissues studied so far. The physiological changes of electrical potential difference observed in frog skin, and also in airways and the gastrointestinal tract of mammals might be related to regulation of the mucous lining on the epithelial surface (GREGER 1996; TYRAKOWSKI *et al*. 1997a & b; KOSIK-BOGACKA & TYRAKOWSKI 2001a), although they are also involved in many other physiological functions (SIMKISS 1988; GREGER 1996; TYRAKOWSKI *et al*. 1997a & b, 1998; KUNZELMAN & MALL 2002; MŁODZIK-DANIELEWICZ & TYRAKOWSKI 2005). It would be interesting to ascertain if in the case of snails similar ionic currents may also be related to the changes of a thin mucous film which covers the whole animal body and also separates the gastropod foot from the substratum. The isolated preparation of the pedal epithelium could then be a convenient model for the study of the interrelation between transepithelial ion trans-

port and mucous film on the epithelial surface (DENNY 1980, 1984, 1989; SIMKISS 1988; DONOVAN & CAREFOOT 1997; PAWLICKI *et al*. 2004).

The aim of the present study was to check if changes of transepithelial electrical potential difference during gentle mechanical stimulation could be measured in isolated pedal epithelium of the snail *Achatina achatina, Lameere* with application of the Ussing method and next to identify provisionally the ion transport pathways generating the transepithelial electrical potential difference by means of the addition of ion transport inhibitors to the stimulation fluid. It was found that changes of transepithelial electrical potential difference occurred during physiological mechanical stimulation in the epithelium of snail foot preparations.

Material and Methods

The experiments were performed on isolated foot specimens of an African snail (*Achatina achatina, Lameere*). In the experiments, the baseline transepithelial electrical potential difference – PD, changes of transepithelial electrical potential difference during stimulation – dPD and the tissue transepithelial resistance were measured with an Ussing apparatus (KOEFOED-JOHANSEN & USSING 1958; TYRAKOWSKI *et al*. 1997b).

Snails were raised in a terrarium on a thin peat layer, they were fed with fresh plants (mainly varieties of cabbage) supplemented with hen eggshell. The epithelial specimens for an *in vitro* study were prepared as follows. Active snails were dissected from their shells, then the muscle layer was partially cut off with a scalpel in order to leave the epithelial layer intact; consequently, experimental specimens of 1-2 mm thickness were produced. Tissues were incubated in Ringer's solution (RH) and after removal of mucous, they were mounted in an Ussing apparatus (surface of the specimen was 1 cm^2). Gentle mechanical stimulation of the epithelial surface of the specimens was performed with the use of Ringer solution in the form of a jet flux from the nozzle connected to a peristaltic pump (2.8 cm^3 of fluid in 1 minute). The stimulation usually lasted for 1 minute.

Chemical stimulation was performed by adding amiloride, bumetanide and DMSO (dimethylsulfoxide) to the Ringer solution. The details of experimental procedures have been already published (TYRAKOWSKI *et al*. 1997a). The measuring equipment consisted of a voltage/current clamp amplifier EVC 4000 (WPI, USA) and the data acquisition system MP 100 (Biopac, USA) connected to a computer. The Ussing chamber was connected to the measuring equipment with agar bridges and two pairs of Ag/AgCl electrodes. The interstitial (laterobasal) side of the specimen was ground and the epithelial (apical) one was usually positive. Each experiment was preceded by equipment control (compensation of electrode potential and of solution resistance in the chamber) and the control of the stimulation procedure with the application of a cellophane membrane instead of tissue. The solutions applied throughout the experiments were (concentrations in mM): RH – Na^+ 147.2; K^+ 4.0; Ca^{2+} 2.2; Cl^- 15.6; Hepes 10; K – Na^+ 4.0; K^+ 147.2; Ca^{2+} 2.2; Cl^- 155.6; Hepes 10. The following test substances were used (concentrations in mM or %): AMI – amiloride 0.01; BUME – bumetanide 0.01; DMSO – dimethyl sulfoxide 1%. Reagents were supplied by Sigma-Aldrich Poznań, Poland. The values of all variables are given as means and standard deviations. For statistical evaluation the data were checked by the Mann-Whitney test and the differences between the means were reported as statistically significant at the value of $P<0.05$.

Results

A total of 60 specimens of foot ventral surface of 28 snails were studied with the electrophysiological method. Electrical parameters of studied tissues were stable throughout the 5 hours of the experiments. Even after 8 hours of the experiment (single observation) the electrophysiological parameters did not decline irreversibly.

The transepithelial electrical potential difference of isolated foot ranged from –6.0 to 10.0 mV under different experimental conditions. The PD value was about 7.2 ± 2.0 mV under control conditions (when Ringer solution without additions was used as experimental fluid). Mechanical stimulation caused transient changes of transepithelial potential difference ranging from –0.7 to 2.0 mV. Under control conditions (RH stimulation) this parameter was 1.13 ± 0.48 mV. Figure 1 presents examples of four different types of time courses of potential difference during experiments. In Figure 1A the PD value increased during the experiment by about 3.53 mV and in Figure 1B it decreased by about 3.56 mV. The variable type of the transepithelial potential difference changes is shown in Figure 1C where fast depolarization (even to –6.0 mV) occurred after a change of liquid in the chamber and then was followed by fast hyperpolarization during the entire experiment. In Figure 1D the stable PD value during the experiment is shown.

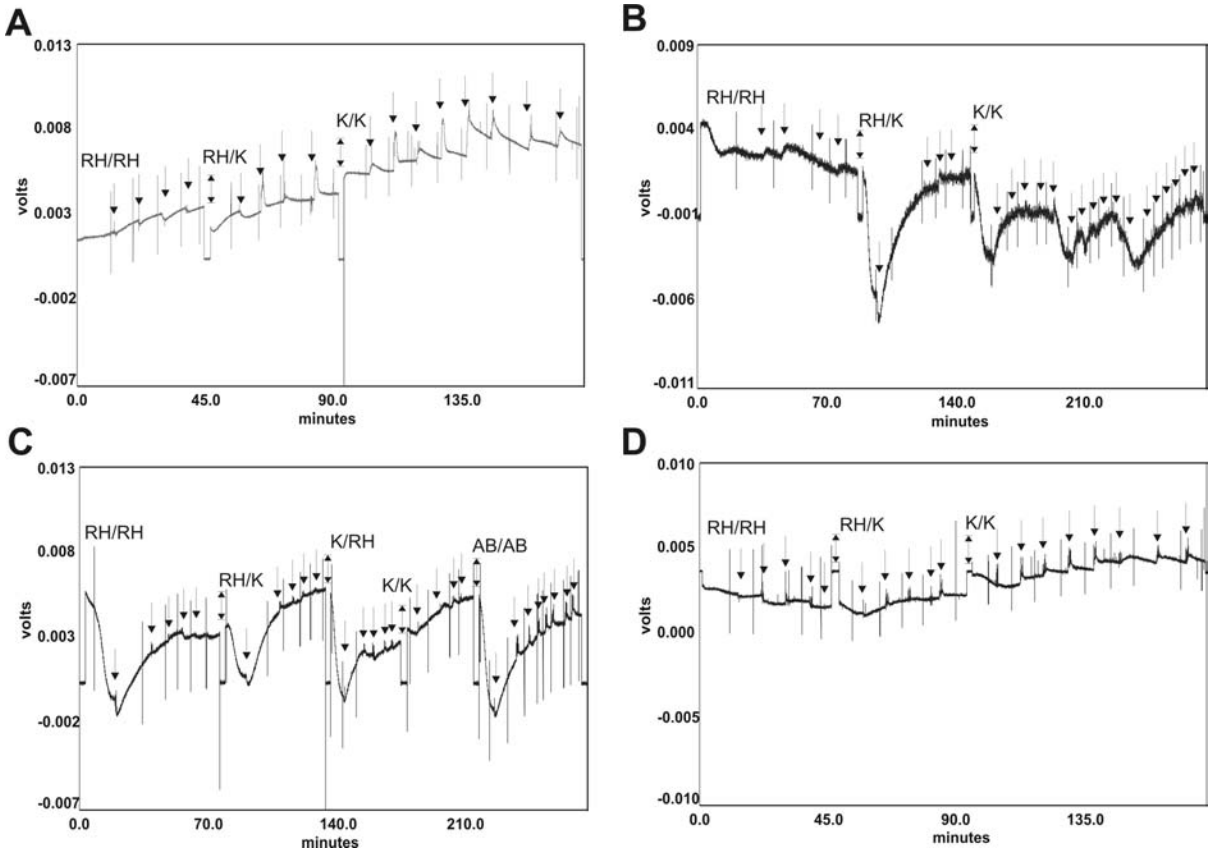

Fig. 1. The time course of electrophysiological study of a single preparation of isolated snail foot pedal surface of *A. achatina* during multiple stimulations. The four different experiments: A, B, C and D are shown. The mechanical or chemical stimulations are denoted by single arrows ↓, the exchanges of bathing fluids (RH – Ringer solution and K – solution with high concentration of K^+) in the both chambers (upper/lower) of Ussing apparatus by double opposite arrows ↕ and the reactions on injection of ± 10 μA current by vertical lines of the graphs.

Table 1

The range of changes of the transepithelial potential difference of the snail foot pedal surface of *A. achatina* during an *in vitro* study

Type of time course (n)	PD_a (mV)	PD_b (mV)	ΔPD (mV)
Increasing (33)	3.4±1.7	6.9±1.5	3.5±1.2
Decreasing (3)	4.7±0.5	1.5±0.6	-3.6±0.9*
Changing (8)	4.4±1.9	5.3±1.2	0.8±0.6*
Stationary (11)	3.8 ±1.2	4.6±1.3	1.0±0.5

The arithmetic means and standard deviations are shown; n – the number of experimental preparations; PD_a , PD_b and ΔPD – the value of the transepithelial potential difference before the first stimulation procedure, after the last one and the difference between PD_b and PD_a, respectively; * denotes a statistically significant difference from the group "Increasing (33)".

Electrophysiological parameters for this group are summarized in Table 1.

The most often occurring responses of the tissue to mechanical and pharmacological stimulations are shown in Figure 2. Mechanical stimulation of the snail foot caused changes in electrogenic ion transport, observed as transient hyperpolarization (transepithelial electrical potential difference become more positive – Figure 2B). When the transepithelial electrical potential difference decreased during stimulation as in Figure 2A the reaction was described as depolarization. When bumetanide, the blocker of the Na^+-K^+-$2Cl^-$ -cotransporter which inhibits the transepithelial chloride ion transport pathway was added to the stimulation fluid, as shown in Figure 2C, transient hyperpolarization with new stationary conditions was set after stimulation. When amiloride and bumetanide

were added to the stimulating fluid (Fig. 2D), so that the sodium and chloride ion transport pathways were inhibited, small, stationary hyperpolarization occurred. The electrophysiological parameters of isolated tissues during stimulations in different conditions are summarized in Table 2. Under control conditions, 22 specimens reacted to mechanical stimulation through a decrease in transepithelial electrical potential difference by about –0.32 ± 0.27 mV, in contrast to 46 specimens which reacted to mechanical stimulation by an increase of PD by about 0.47 ± 0.34 mV. Changes of transepithelial electrical potential difference after mechanical stimulation in the presence of bumetanide or amiloride and bumetanide were 0.87 ± 0.34 and 0.79 ± 0.28 mV, respectively. The single tissue responses to stimulation under conditions of higher extracellular potassium ion concentration

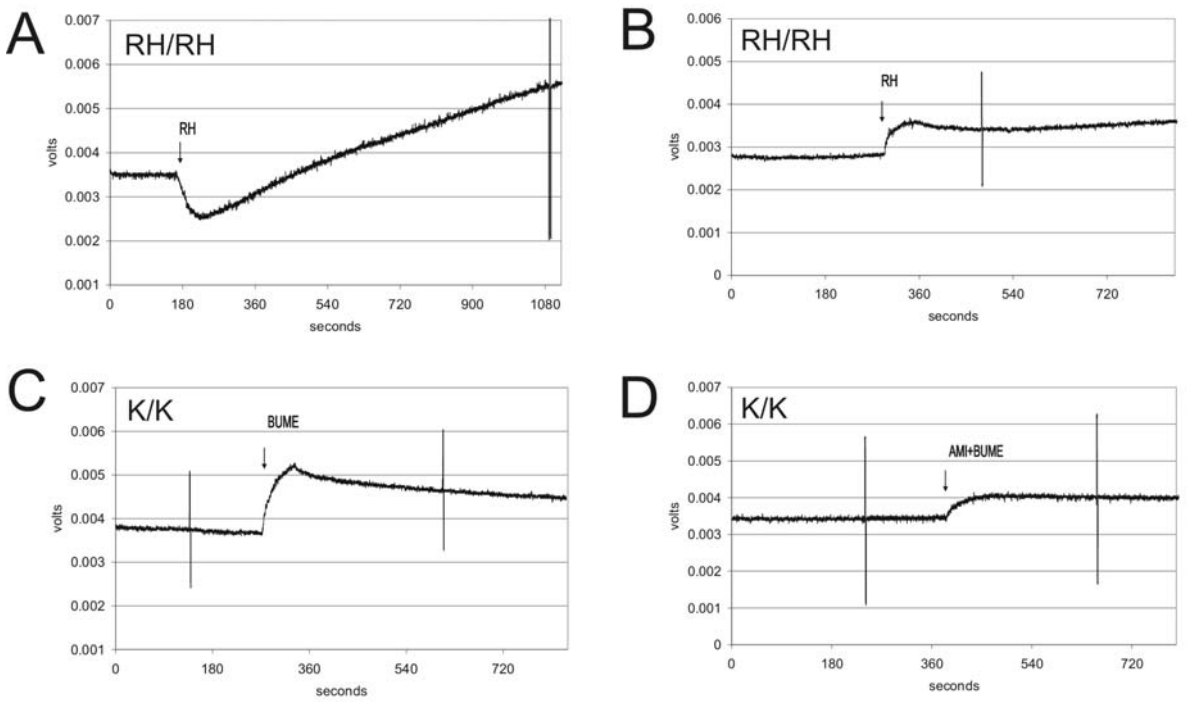

Fig. 2. The effects of the mechanical stimulation under control conditions and in the presence of inhibition of transepithelial Cl^- transport by bumetanide and Na^+ transport by amiloride on the transepithelial potential difference of isolated snail foot pedal surface of *A. achatina*. The single experiments are shown. The bathing fluids in the both chambers (upper/lower) of Ussing apparatus are denoted as follows: Ringer solution – RH and solution with high concentration of K^+ - K. The stimulation by gentle rinsing is denoted by arrows: with Ringer solution (RH) – A and B, in the presence of bumetanide (BUME) – C and in the presence of amiloride and bumetanide (AMI+BUME) – D. The vertical lines on the graphs are the responses on the stimulation with ± 10 μA current.

Table 2

The range of changes of the transepithelial potential difference of the snail foot pedal surface of *A. achatina* during mechanical stimulation and under conditions of inhibited Na^+ and Cl^- transport

Solution/reaction on stimulation (n)	PD_a (mV)	dPD (mV)	PD_b (mV)	R Ω*cm^2
RH/depolarization (22)	3.4±1.8	-0.3±0.3	3.2±1.5	260±74
RH/hyperpolarization (46)	4.2±1.3	0.5±0.2*	4.5±1.2	251±81
BUME/hyperpolarization (29)	7.9±1.9	0.9±0.3*	8.4±2.1	222±47
AMI+BUME/hyperpolarization (27)	7.6±1.9	0.8±0.3*	8.1±1.9	214±38

The arithmetic means and standard deviations are shown; n – the number of experimental reactions; PD_a, PD_b and dPD – the value of the transepithelial potential difference before and after the stimulation procedure, and the difference between the maximum value during stimulation and control before stimulation, respectively; experimental conditions: RH – Ringer solution was used as bathing and stimulation fluid, BUME and AMI+BUME – the bathing fluid with high K^+ concentration and stimulation fluid with bumetanide or amiloride and bumetanide, respectively, were applied; * denotes a statistically significant difference from the group "RH/depolarization (22)".

(with solution denoted K) were smaller than under control conditions as shown in Fig. 3 A. During stimulation in the presence of bumetanide (Fig. 3 D) only the second phase of the reaction was stopped, i.e. after the initial change, the tissue did not return to the control value. The variety of tissue reactions to the same kind of impulse are illustrated in Figures 3A, C, and 3E, F. The basic electrophysiological parameters for the whole group are illustrated in Tab. 3, with a note of statistically significant differences between reactions.

Figure 4 presents the sequence of reactions of snail foot preparations to the series of mechanical stimulations in the presence of different pharmacological substances which modified these reactions. In relation to control conditions (RH) after addition of DMSO, bumetanide, amiloride or both substances, the ionic currents responsible for the return of PD to the prestimulatory level were markedly inhibited in opposition to relatively well preserved hyperpolarization currents. This pharmacological test enabled a preliminary estimation of specific ion currents which take part in the reactions of the tissue to mechanical stimulation; the tests lasted about 40 minutes. A summary of these experiments are in Table 4 with highlighted significant differences between the reactions.

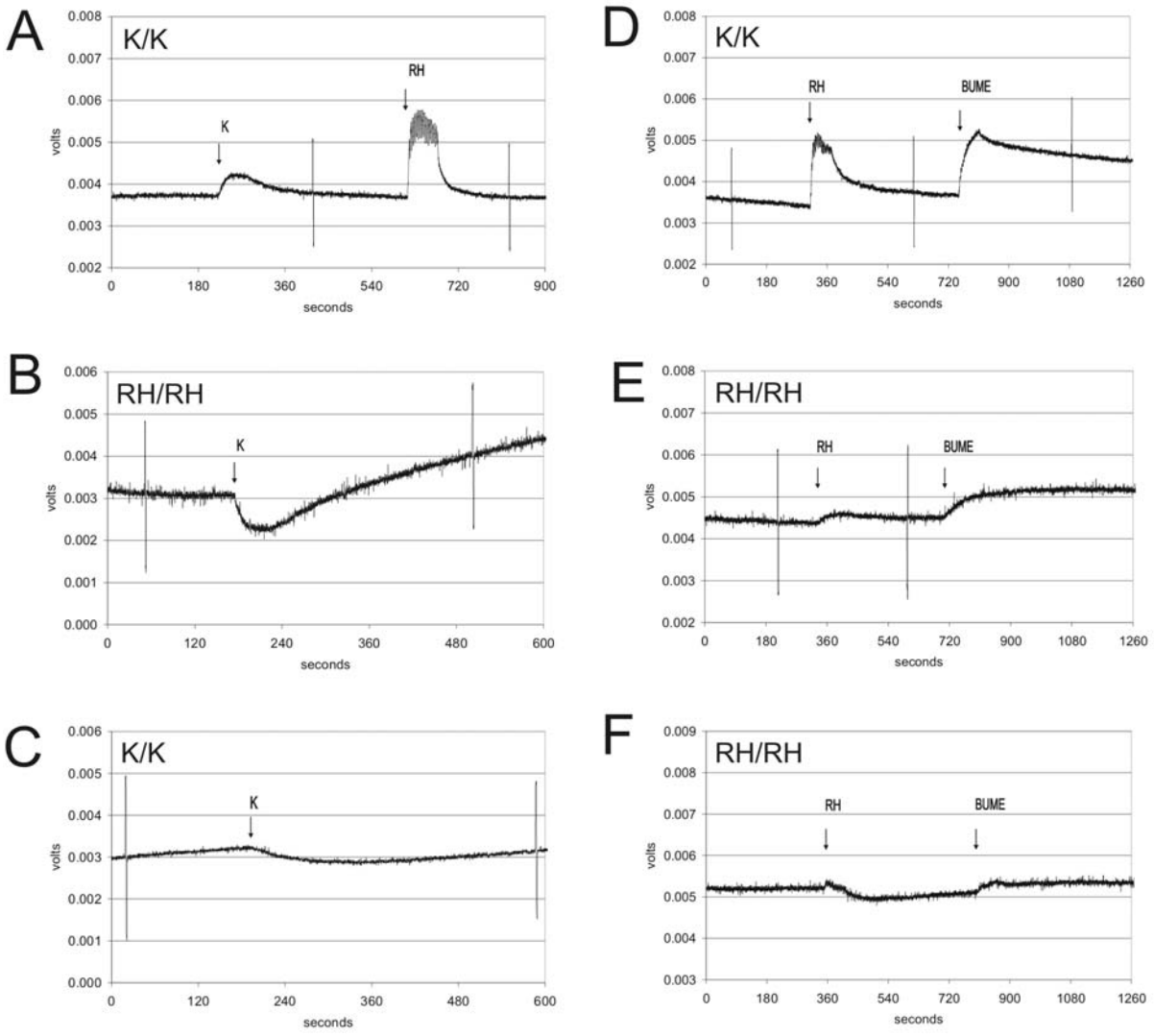

Fig. 3. The effects of the augmentation of the K^+ transport by higher potassium ion gradient and of the inhibition of the Cl^- transport by addition of bumetanide on the changes of potential difference of isolated snail foot pedal surface of *A. achatina* evoked by mechanical stimulation. The single experiments are shown. Experimental conditions: the bathing fluid with high K^+ concentration (K) was applied in A, C and D and Ringer solution (RH) in B, E and F; the stimulation fluid in the both chambers (upper/lower) of Ussing apparatus with high K^+ concentration was denoted by K, with bumetanide by BUME and Ringer solution by RH. The vertical lines on the graphs are the responses on the stimulation with ± 10 μA current.

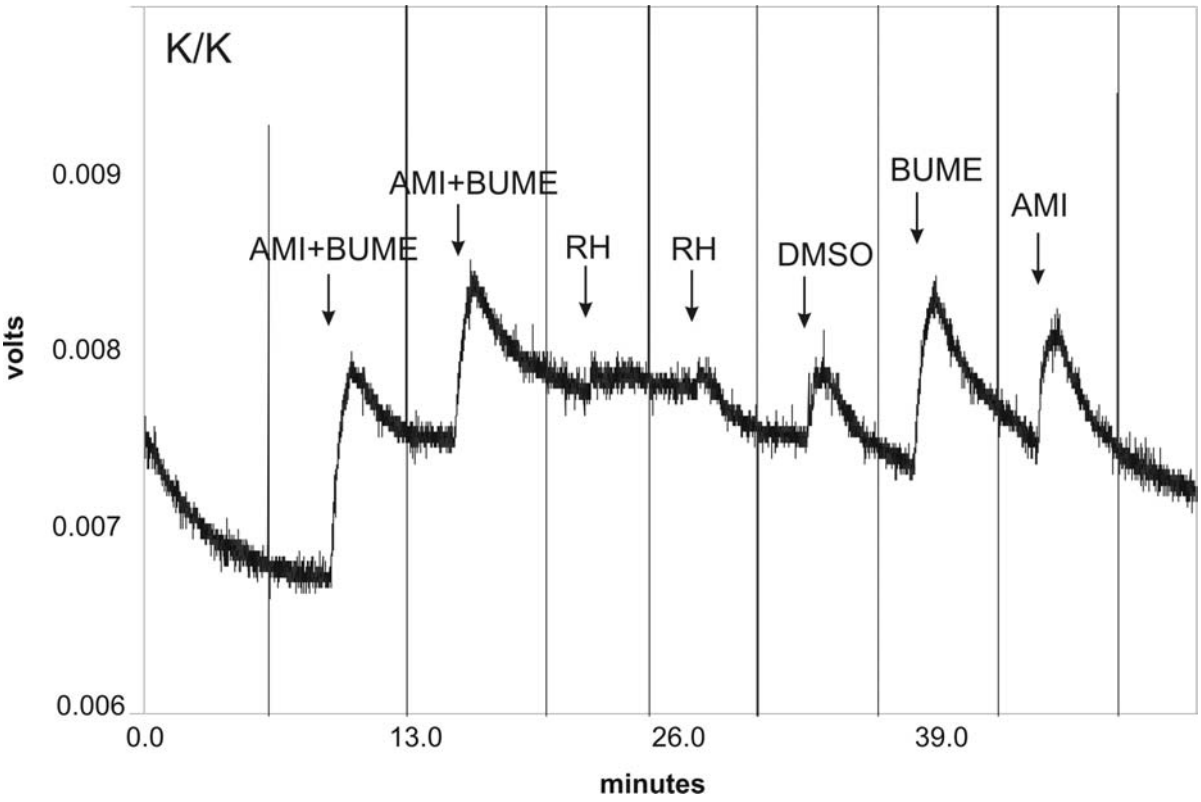

Fig. 4. The successive effects of the sequence of mechanical stimulations with the irritant substance – DMSO, in the presence of inhibition of Na^+ or/and Cl^- transport with amiloride – AMI and bumetanide – BUME and both – AMI+BUME, respectively, on the changes of potential difference of isolated snail foot pedal surface of *A. achatina*. The single experiment is shown. The stimulation by gentle rinsing is denoted by an arrow and the bathing fluid in the both chambers (upper/lower) of Ussing apparatus with high K^+ concentration by K. The vertical lines on the graphs are the responses on the stimulation with ± 10 μA current.

Discussion

The epithelium, which forms the most external part of the gastropod integument and actively cooperates with the nervous, muscular, immune and hormonal elements, fulfills many physiological and control functions. The integration of different, sometimes antagonistic functions, is realized by an internal control system closely associated with the integument and different from the reflexes of the snail nervous system. The reactions of this system to mechanical impulses and pharmacological modification are described in this paper.

The experimental model characteristics

The application of isolated pedal specimen of the snail foot in the USSING apparatus showed that the electrical potential difference of this preparation changed from less than -6.0 to more than 10.0 mV (Fig. 1, Table 1 & 2). Under the influence of different stimuli, i.e. mechanical (gentle rinsing), chemical (changes of ions concentration) and pharmacological (application of ion transport inhibitors) transient changes of the potential difference (dPD) were evoked, ranging from about –0.7 to almost 2.0 mV (Fig. 2, Table 1 & 2). These results suggest that the epithelium of the snail foot of *A. achatina* dynamically regulated the electrogenic ion currents. The positive sign of the transepithelial electrical potential of the outer face of snail integument, in relation to the inner inside as ground, was already observed in the seventies of the last century and then repeatedly confirmed (ISTIN & FOSSAT 1972; ENYIKWOLA & BURTON 1983; SIMKISS 1988).

Epithelial cells of snail foot are tightly connected and from the apical site they have cilia and microcilia (DENNY 1984; SIMKISS 1988; FACCIONI *et al*. 2004). This epithelium regulates the quantity of water in the mucus, excretion of metabolites, and transepithelial ion fluxes; it also takes part in locomotive activity as the snail is isolated from a substratum by a mucous layer which alternately acting as glue or lubricant (MORTON 1964, 1988; ROBERTSON 1964; DENNY 1980, 1984, 1989; ENYIKWOLA & BURTON 1983; SIMKISS 1988; CHIASSON *et al*. 1994; GREGER 1996; FACCIONI *et al*. 2004).

The method of mechanical stimulation by means of gentle rinsing of the epithelial surface of the preparation by a medium from a nozzle connected with a peristaltic pump seems to reflect physiological stimulation of the nervous mechanorecep-

tors during movement. The well preserved structure of the tissue and the adequate stimulation both allowed for the reaction to gentle rinsing to be evoked as a change in electrical potential. Consequently, this suggests that the interaction between mucus and epithelial surface occurs through ion transport changes (DENNY 1984) and is controlled at two levels: cellular and neural (ENYIKWOLA & BURTON 1983). It is hypothesized that the physiological function of the transient transepithelial currents consists of their influence on negatively charged mucus molecules (TYRAKOWSKI 1997b; MŁODZIK-DANIELEWICZ & TYRAKOWSKI 2005).

In the nervous system of the mollusks, afferent (sensory) neurons, interneurons and efferent (motor) neurons exist; all of them control different body functions (GRENON & WALKER 1980; BARNES *et al*. 1994; BREIDBACH & KUTSCH 1995; DONOVAN & CAREFOOT 1997; PIVAROV & DROZDOWA 2002a). Many different transmitters, such as acetylocholine, dopamine, serotonin, histamine, gammaaminobutric acid, nitrogen oxide and others, play a role in the activity of nervous cells (BARNES *et al*. 1994; BREIDBACH & KUTSCH 1995; DONOVAN & CAREFOOT 1997; NÄSSEL 1999; LEAH DEVLIN 2001, MICHAELIDIS *et al*. 2002). Some of these are tachykinin-related peptides (TRP), which are released from sensory endings and, most likely, act in mollusks as neurokinins do in mammals (NÄSSEL 1999). The opening and closing of ion channels in epithelial cells (reflected by changes of the electrical potential), controlled by (nervous) cells, are mediated by specified neuromediators (AHEARN *et al*. 1994; BARNES *et al*. 1994; YOSHIDA & KOBAYASHI 1995; WATANABE *et al*. 1998). It is plausible that differences in the concentration of these neuromediators, which are regulated locally, are the main reasons for the differences observed between the specimens from the same animal.

In the course of every experiment, before and after mechanical stimulation, the electrical resistance was measured. This parameter reflects the functional integration of tight junctions between cells, marks the viability of cells and the physiological epithelial permeability for ions (HOYLE 1964; FUNASE *et al*. 1993). It was shown in this study that this parameter did not change during experiments (Table 2, 3 & 4).

Although Hans Ussing and coworkers developed the electrophysiological method of studying transepithelial electrical potential in isolated epithelial tissues in the sixties of the former centenary (KOEFOED-JOHANSEN & USSING 1958), the application of the modified Ussing chamber and modern measurement techniques resulted contemporarily in the discovery of new transport pathways and complex characteristics of the transepithelial ion currents during different physiological events.

The problems of integration of electrogenic ion currents with other physiological activities can be addressed with the already described Ussing method or by transepithelial measurement *in vivo*.

Table 3

The influence of changes of the potassium ion gradient and the inhibition of the Cl^- transport by addition of bumetanide on the changes of potential difference of isolated snail foot pedal surface of *A. achatina* evoked by mechanical stimulation

Bathing fluid\stimulation fluid (d/h), (n)	PD_a (mV)	dPD (mV)	PD_b (mV)	R Ω*cm^2
K/K, (h), (61) a	5.7±1.9	0.4±0.3	5.9±2.2	192±57
K/RH, (h), (61)	5.9±1.9	1.1±0.6*	6.4±2.2	200±57
RH/K, (d), (13) b	3.1±1.1	-0.4±1.2*	2.8±1.3	219±57
K/K, (d), (5) c	3.1±1.5	-0.2±0.03*	3.1±1,5	230±40
K/RH, (h), (25) d	7.2±1.9	1.2±0.5	7.5±1.8	250±39
K/BUME, (h), (25)	7.9±1.8	1.0±0.5	8.6±1.6*	237±64
RH/RH, (h), (7) e	4.8±1.4	0.3±0.1	5.0±1.4	210±22
RH/BUME, (h), (7)	4.9±1.4	0.2±0.1	5.2±1.5	212±34
RH/RH, (d), (7) f	4.2±1.5	-0.2±0.1	4.0±1.4	274±78
RH/BUME, (h), (7)	4.3±1.2	0.2±0.1	4.4±1.3	210±20

The arithmetic means and standard deviations are shown; d/h – denotes the type of reaction on stimulation: depolarization or hyperpolarization, respectively; n – the number of experimental reactions; PD_a, PD_b and dPD – the value of the transepithelial potential difference before and after the stimulation procedure, and the difference between the maximum value during stimulation and the control before stimulation, respectively; R – transepithelial electrical resistance; experimental conditions: RH – Ringer solution, K – solution with high K^+ concentration, BUME – the solution with addition of bumetanide; a, b, c, d, e and f – correspond to the Fig. 3. A, B, C, D, E and F, respectively; * denotes a statistically significant difference from the group K/K, (h), (61) for the variable dPD or from the variable PD_a for the variable PD_b.

Table 4

Effects of the sequence of mechanical stimulations in the presence of an irritant substance – DMSO and during inhibition of Na^+ and/or Cl^- transport on the changes of potential difference of isolated snail foot pedal surface of *A. achatina*

Experimental conditions (n)	PD_a (mV)	dPD (mV)	PD_b (mV)	R Ω*cm^2
RH (32)	7.2±2.0	1.1±0.5	7.6±2.0	248±31
DMSO (26)	7.2±2.0	1.0±0.4	8.3±1.8*	212±47
BUME (32)	7.9±1.9	1.0±0.5	8.7±1.8*	261±33
AMI (29)	7.5±1.9	0.7±0.2*	7.7±2.0	219±48
AMI+BUME (28)	7.6±1.9	0.8±0.3*	8.2±2.0	214±38

The arithmetic means and standard deviations are shown; n – the number of experimental reactions; PD_a , PD_b and dPD – the value of transepithelial potential difference before and after the stimulation procedure, and the difference between maximum value during stimulation and the control before stimulation, respectively; R – transepithelial electrical resistance; experimental conditions: RH – Ringer solution, DMSO – solution with addition of dimetyl sulfoxide, BUME, AMI and AMI+BUME – solutions with addition of bumetanide, amiloride and both, respectively; experimental conditions correspond to the Fig. 4; * denotes statistically significant difference from the group "RH (32)".

Ion currents evoked by mechanical stimulation

The variable described as change of the potential difference after mechanical stimulation (dPD) is a function of time and reflects momentary algebraic sums of transepithelial ion currents from onset of stimulation to the achievement of a new stationary stage by the tissue. The dPD-function has two distinct phases: the first occurs during stimulation and usually, but not without exceptions, was seen as hyperpolarization; the second was the return of the PD to the stationary value and was usually seen as depolarization.

In some cases the opposite course was observed – depolarization preceded hyperpolarization and then the tissue achieved a new likely stationary stage. The increase of the positive transepithelial potential difference results from positive ion transport through the apical cell membranes (or from a negative ion transport in the opposite direction) and the decrease of the parameter is a result of the secretion of negative ions (or absorption of positive ions).

The presence of transporting proteins for different ions in a variety of snail cells have already been shown and could be expressed in epithelial cell (ROBERTSON 1964; ISTIN & FOSSAT 1972; SIMKISS 1988; FUNASE *et al*. 1993; ILIEV & MARINO 1993; AHEARN *et al*. 1994; BARNES *et al*. 1994; BREIDBACH & KUTSCH 1995; GREGER 1996; DONOVAN & CAREFOOT 1997; TYRAKOWSKI *et al*. 1998; PIVAROV & DROZDOWA 2002a, b; FACCIONI *et al*. 2004).

The following positive ions (cations): potassium, sodium, calcium and hydrogen, and negative (anions): chloride and bicarbonate should be taken into consideration as possible current carriers. The contribution of potassium ions was studied by decreasing the concentration gradient between the cytoplasm and the external environment of epithelial cells, by application of increased concentration of potassium ions in the USSING apparatus bathing or stimulation fluid (Fig. 3 & Table 3). The changes of potential difference were influenced by potassium ion concentration (Fig. 3A – RH, also Table 3 – 1) and were larger when the concentration gradient increased. This suggests that the hyperpolarization of the epithelium after mechanical stimulation is a result of increased potassium ion secretion during the opening of the apical potassium channels. The contribution of chloride ion secretion in the second (depolarization) phase of the dPD-reaction was shown by the addition of bumetanide to the experimental fluids, which virtually eliminated the return of PD to the control prestimulatory level (Fig. 2C, 3D, E & F, Table 2 and 3). Although the potassium and chloride ion transporters are mainly responsible for dPD reaction at the snail *A. achatina*, after blocking both of these currents there is still a small, significant reaction left which waits for further study. Probably bicarbonate is reabsorbed in the hyperpolarization and the same happens with sodium ions during depolarization.

The inhibition of the depolarization by DMSO resulted from the influence of these reagents on the release of TRP peptides, which corresponds with the opinion that DMSO is an afferent fibre stimulant.

The stationary (without stimulation) transepithelial potential difference – PD depends on similar ion transport pathways as the already described reactions during stimulation (ROBERTSON 1964; ISTIN & FOSSAT 1972; SIMKISS 1988; FUNASE *et al*. 1993; ILIEV & MARINO 1993; AHEARN *et al*. 1994; BARNES *et al*. 1994; BREIDBACH & KUTSCH 1995; GREGER 1996; DONOVAN & CAREFOOT

1997; TYRAKOWSKI 1998; PIVAROV & DROZDOWA 2002a, b).

From these studies it is concluded that the prevailing transepithelial electrogenic transport system in this snail species is that of K^+ and Cl^- ions.

The importance of potassium ion transport for the electric phenomena on epithelial surface is stressed by: (1.) the positive sign of the PD (it may be produced by K^+ secretion), (2.) the insensitivity to amiloride inhibition (it cannot be Na^+ transport) and 3. the depolarization by augmented K^+ concentration in experimental fluids (so the driving force for K^+ current is diminished or simply eliminated) as shown in Figures and Tables (Fig. 2D & Fig. 4, Table 2 AMI + BUME, Tab. 4 AMI and AMI+ BUME and Fig. 2D, Table 3-K).

The final conclusion is that the changes of transepithelial potential difference of the pedal surface of the snail's foot are related to physiological stimuli (probably they are involved in the adhesive locomotion of the species) and they are under the control of the part of the nervous system where the TRP peptides are the transmitters.

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