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Short Note

The Bi-phased Course of Electrophysiological Response of Isolated Snail Intestine on Mechanical Stimulation

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The transepithelial potential difference and changes of diameter of isolated snail intestine as index of its motility were studied in immersed bath in control conditions and after gentle stimulation by 60 seconds of washing of the intestinal lumen. Immediate depolarization and 20% augmentation of the lumen were observed during the stimulation. After stimulation, additional transient depolarization of the transepithelial potential difference and gradual diminution of intestine lumen back to control values over a period of 20 minutes occurred. The immediate reaction was greatly influenced by the presence of sodium or chloride ion transport inhibitors, however, the late phase of the response was not. It is hypothesized that changes of transepithelial electrogenic ion transport and of intestinal motility during the stimulation mirror the inflow of intestinal content and after completion of stimulation may be related to its storage.

Key words: Electrophysiology, transepithelial potential difference, ion transport, intestine, snail, *Achatina achatina*.

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Movement on an epithelial surface, as related to the dislocation of intestinal content and clearing of mucous plugs from airways during coughing in mammals or to amphibian and snail integument during locomotion or defensive reflexes (LUCHTEL & DEYRUP-OLSEN 2001; SIMKISS & WILBUR 1977), invariably evokes transient changes in the transepithelial potential difference (GRECZKO & TYRAKOWSKI 2001, KOSIK-BOGACKA *et al*. 2000, 2003; KOSIK-BOGACKA & TYRAKOWSKI 2001, 2002; MŁODZIK-DANIELEWICZ & TYRAKOWSKI 2005; TYRAKOWSKI *et al*. 1997, 1998a, 1998b, 2006).

It is unknown whether mechanical stimulus evokes potential difference changes in snail intestine and the physiological, neuronal and humoral mechanisms of this reaction have not been addressed (DIMITRIADIS 2001; ROLDAN & GARCIA-CORRALES 1988).

In this article a preliminary account on this problem is presented with special emphasis on the correlation between electrophysiological variables and intestinal motility. The study was performed in an immersion bath with continuous measurement of electrophysiological parameters and also

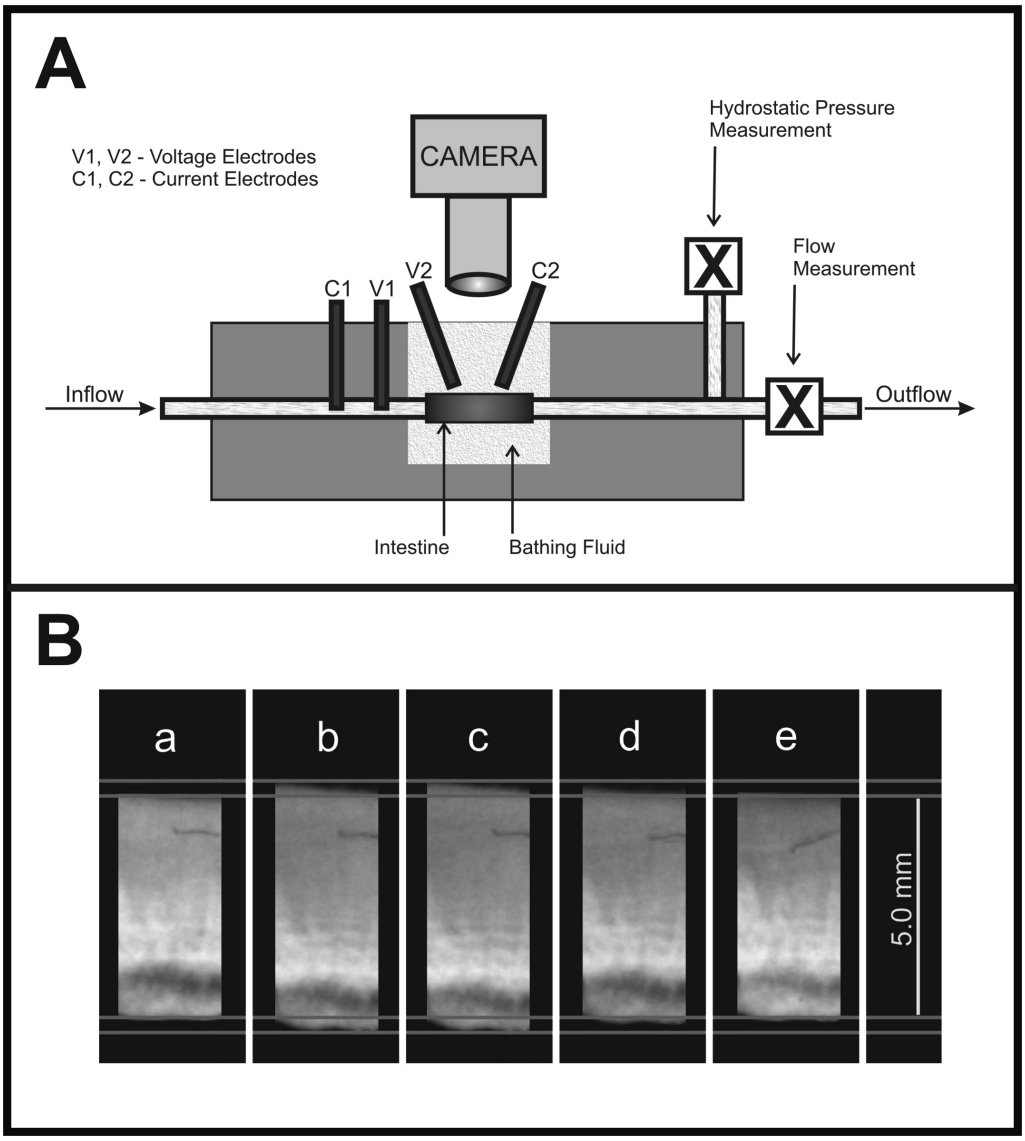

Fig. 1. The changes in diameter of isolated snail intestine as a reaction on gentle mechanical stimulation. A – diagram of the experimental setup for simultaneous recordings of electrophysiological variables and changes in intestine diameter as an index of its motility. B – The series of photos of the central part of isolated snail intestine in different time points during the course of the experiment. a – before stimulation, b – at 30 s of stimulation, c – at 60 s of stimulation, d – at maximum value of depolarization after stimulation, e – at the end of the response.

with continuous recording of intestinal diameter by means of a camera.

Material and Methods

The experiments were performed on isolated intestine from Giant African Snail (*Achatina achatina, Lameere*). The snail shell was gently halved with an electrical saw and the palium was dissected. The gastrointestinal tract of the snail was exposed and about 10 mm of intestine was excised. The intestinal content was removed by gentle rinsing with Ringer solution (RH) and the specimen was mounted and fixed with surgical silk threads on cannulae in the immersed bath chamber. The chamber (Fig. 1A) of inner size 30x20x15 mm was made of plexiglass and was equipped on opposite inner walls with inlet and outlet cannulae for intraluminal perfusion. The distance between cannulae was 6 mm.

The hydrostatic pressure and output of fluid were controlled during the whole experiment. The voltage V1 and current C1 electrodes were connected with intraluminal fluid and respective V2 and C2 electrodes with extraluminal fluid. The electrodes V1-V2 and C1-C2, respectively, were connected to a system consisting of a preampli-

fier, amplifier (EVC4000, WPI, USA) and the data recording module (MP-100, Biopac, USA, with the program AcqKnowledge 3.8.1).

An image of the intestine from a CCD camera (DFK41 AV02.AS, The Imagingsource, Germany, with lens CCTV 5-50 mm F/1.8, Pentax) was digitally recorded (JPG files) by using the IC Capture.AS 2.0 program.

Experiments consisted of gentle washing of intestinal lumen without or in the presence of inhibitors of transepithelial transport. In control conditions Ringer solution was used as intraluminal and extraluminal fluid. Stimulation included an intraluminal flux of Ringer solution from a peristaltic pump during 60 seconds under 22 mm of hydrostatic water pressure and of 10 ml output. Impulses consisted of gentle movement of fluid and small tension on the intestinal wall under hydrostatic pressure. Changes of transepithelial electrical potential difference in the control period (PD), during stimulation (dPD) and during 20 minutes after stimulation (dPD_{max} – maximum value of PD), PD_{end} – value of stabilization of PD) were measured. The electrical resistance (R) was measured before and after each stimulation procedure. Changes in diameter of intestine before, during and after stimulation were measured on photos in defined time points.

The solutions used were: Ringer solution (RH: Na^+ 147.2 mM, K^+ 4.0 mM, Ca^{2+} 2.2 mM, Cl^- 155.6 mM, Hepes 10.0 mM), RH with the addition of 100 μM amiloride (AMI), RH with the addition of 100 μM bumetanide (BUME), RH with the addition of 100 μM amiloride and 100 μM bumetanide (AMI+BUME).

The results were presented as arithmetic means ± standard deviations. The Student *t*-test was used to determine the significance of difference between means with a probability value of <0.05 (P<0.05).

Results

A total of 10 intestines from different snails were studied. Changes of intestinal diameter before, during and after mechanical stimulation are shown in Table 1 and Figure 1B.

Table 1

Changes in diameter of isolated snail intestine as a reaction to gentle mechanical stimulation

Conditions of measurements	Diameter of intestine (mm)
Before stimulation (n=32)	5.0±0.6
At 30 s of stimulation (n=32)	5.5±0.5*
At 60 s of stimulation (n=32)	5.6±0.6*
At maximum value of depolarization after stimulation (n=32)	5.3±0.6*
At the end of response (n=32)	5.1±0.6*

The stimulus was the flux of bathing fluid from a peristaltic pump lasting 60 seconds, of 0.17 ml/s output and under 22.0 mm water hydrostatic pressure. The whole reaction lasted about 20 minutes with maximum depolarization (see Fig. 2) about 6 minutes after stimulation. The results are presented as arithmetic means ± standard deviations.
* – significantly different in comparison to the conditions "before stimulation" at P<0.05.

Table 2

The course of transepithelial potential difference and changes of electrical resistance of isolated snail intestine during and after gentle washing of its luminal surface during 60 seconds

Experimental conditions	The immediate reaction			The late reaction			
	PD [mV]	dPD [mV]	R [Wcm^2]	dPD_{max} [mV]	t_{max} [min]	PD_{end} [mV]	t_{end} [min]
RH (n=10)	-0.10±0.99^a	1.78±0.72^b	1460.8±295.2^c	3.07±1.16^d	7.1±3.1^e	1.21±1.13^{*a}	20.9±9.6^f
AMI (n=10)	1 .36 ±1.12^{*a}	0.91±0.72^{*b}	1663.2 ±332.0^{*c}	2.95±1.42	6.0±1.5^{*e}	1.34±0.97	27.5±10.9^{*f}
BUME (n=10)	1.30±0.97^{g,*a}	0.83±0.34^{*b}	1847.5±302.4^{*c}	3.06±1.28	5.3±1.4^{*e}	1.97±0.83^{*e,*g}	18.6±6.3
AMI+BUME (n=10)	1.8±0.83^{h,*a}	0.42±0.46^{*b}	1717.0±221.0^{*c}	3.11±1.38	6.1±1.8	2.10±0.87^{*e,*h}	19.3±7.0

Changes during the stimulation or after its completion are distinguished as immediate or late reactions, respectively. Washing was performed with Ringer solution only – RH, with addition of amiloride – AMI, with addition of bumetanide – BUME, and with addition of both substances – AMI+BUME. PD – transepithelial potential difference (mV), dPD – the difference between maximum PD value during stimulation and in control condition, R – electrical transepithelial resistance (Ωcm^2), dPDmax – the difference between maximum PD value after stimulation and in control condition, PDend – PD value at the end of late reaction, tmax – the time from the start of the stimulation to occurrence of dPDmax, tend – the time from the start of the stimulation to the occurrence of PDend. The results are presented as arithmetic means ± standard deviations.
* – significantly different in comparison to the conditions specified by letters a, b, c, d, e, f, g, h at P<0.05.

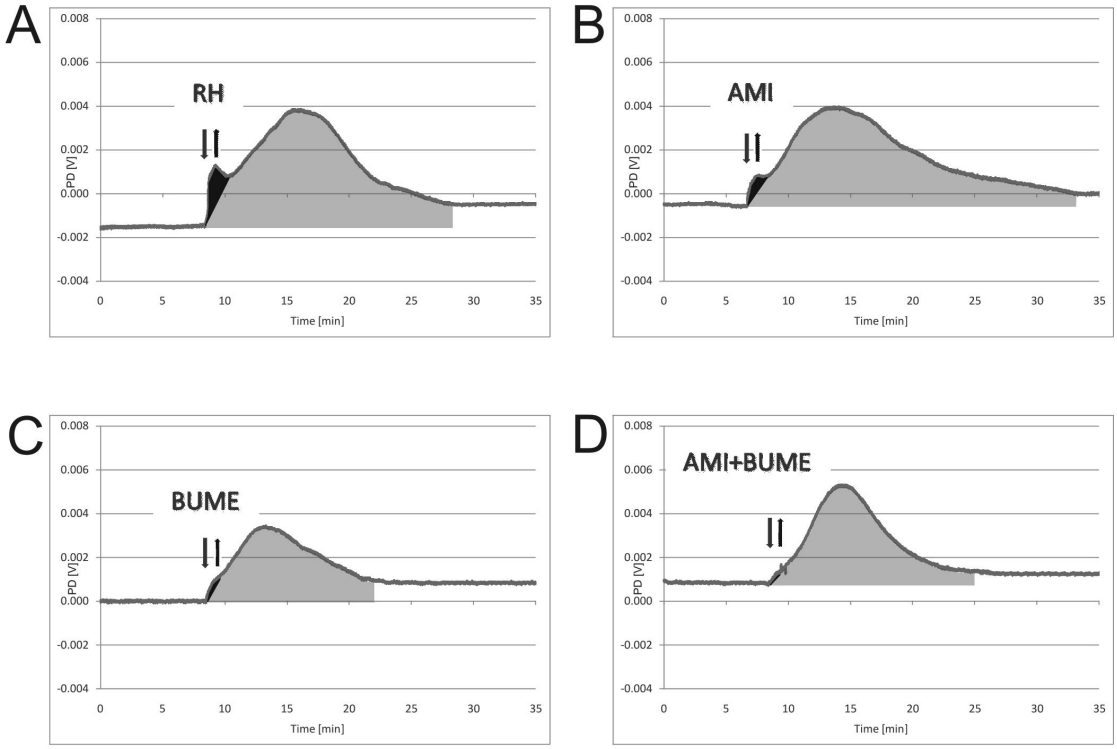

Fig. 2. The course of transepithelial potential difference of isolated snail intestine during and after gentle washing of its luminal surface during 60 seconds. The area under the curve during stimulation is shaded and is distinguished as the immediate reaction. The area after completion of stimulation is hatched and termed the late reaction. Washing was performed with Ringer solution (RH) only – A, with addition of amiloride (AMI) – B, with addition of bumetanide (BUME) – C, and with addition of both substances (AMI+BUME) – D. The single experiment is shown.

Mechanical stimulation, i.e. the gentle washing of the internal surface of the isolated intestine caused an augmentation of the cross section area of about 20% at the end of stimulation. The cross section returned to the control value after about 20 minutes.

In Table 2 the influence of mechanical stimulation on electrophysiological parameters for the whole experimental group and in Figure 2 the course of potential difference changes during a single experiment are shown. Simultaneously with the augmentation of intestinal diameter, the transepithelial potential difference changed about 1.7 mV during washing with Ringer solution only. When inhibitors of sodium – amiloride, or chloride ion transport – bumetanide, or both were added, the changes were 0.9, 0.8 or 0.4 mV, respectively.

After stimulation, the transepithelial potential difference was additionally augmented gradually by some 3 mV during the next 6 minutes and then slowly returned to control values during the following 10 minutes.

The quantitative relationships between electrogenic transepithelial ion transport changes during or after mechanical stimulation were calculated as the ratios of the specified areas under function of transepithelial potential difference in time to the area of the whole reaction (Table 3).

Table 3

Quantitative relationship between immediate and late phases of electrogenic transepithelial ion transport changes after mechanical stimulation of lumenal surface of isolated snail intestine

Experimental conditions	The immediate reaction	The late reaction
RH (n=10)	4.0%	96.0%
AMI (n=10)	1.7%	98.3%
BUME (n=10)	0.7%	99.3%
AMI+BUME (n=10)	0.3%	99.7%

The areas under function of transepithelial potential difference in time during stimulation and after its completion for immediate and late reaction, respectively, were calculated and presented as percent of the area of the whole reaction. Legend – see Table 2.

Discussion

The whole isolated snail intestine was studied in immersed bath. Intestinal motility was usually monitored by force transducers in this setting (compare KENAKIN 1984; FARMER & COLEMAN 1970; VAN NEUTEN & FONTAINE 1976; DOERR-KREMER 1991) but contemporary image analysis computer programs offer the possibility of recording this activity via a camera connected to a computer as performed in this study. The transepithelial potential difference was measured according to the Ussing method (KOEFOED-JOHNSEN & USSING 1958) by means of electrodes connected to a voltmeter and a data acquisition system. The tissue-bath system was equipped with a stimulation device which consisted of a connection to a peristaltic pump and produced a flux of bathing fluid on the luminal intestinal surface.

The setup allows for the gentle washing of luminal surfaces, simultaneous measurements of transepithelial potential differences and observation of changes in intestinal motility.

From experimental results a bi-phased response of snail intestine on luminal mechanical stimulation has emerged. There were some changes during stimulation and these were clearly different from the ones occurring later. The first part of the response is called the immediate reaction, and the second is termed the late reaction.

The immediate reaction lasted one minute and was dependent on the continuous presence of mechanical stimulation. The late reaction lasted 20 minutes, it was initiated by stimulation but then proceeded spontaneously.

The immediate reaction was greatly influenced by the presence of sodium or chloride ion transport inhibitors – amiloride and bumetamide, respectively, but the late phase of the response was not influenced.

It is hypothesized that the observed changes of transepithelial electrogenic ion transport and of intestinal motility during these experiments mirror the physiological activity of the intestine *in vivo*.

The small depolarization of about 1.0 mV and 25% augmentation of intestinal lumen during the immediate reaction could be explained as the physiological equivalent of the inflow of intestinal content. The late reaction with much greater depolarization and gradually decreasing diameter of the intestine could represent the phase of accumulation of the content.

The transepithelial ion transport processes of the intestine, physiologically, consist of secretion and reabsorption and from the electrophysiological point of view of electroneutral or of electrogenic phenomena. From the presented experiment it is concluded that only immediate transport processes depend on sodium and/or chloride ions, as both are diminished by the addition of the specific inhibitors – amiloride and bumetanide.

Ion transport during the late reaction was not influenced by amiloride and/or bumetanide so only a preliminary suggestion that it represents potassium ion secretion, is possible.

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