Medical and Biological Sciences, 2013, 27/2, 27-31

ORIGINAL ARTICLE / PRACA ORYGINALNA

Wojciech Pawłowicz

MODEL STUDY OF EPITHELIAL MOTILITY

MODELOWE BADANIA AKTYWNOŚCI RUCHOWEJ NABŁONKA

Department of Pathobiochemistry and Clinical Chemistry, Collegium Medicum, Nicolaus Copernicus University,
M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland
Head of Department: Professor Tomasz Tyrakowski

S u m m a r y

One of the oldest achievements of human thought is the use of plants and plant extracts in therapeutics. Drugs of plant origin are characterized by multi-effects. In recent years, much interest in medicinal plants containing a mixture of biologically active substances with antimicrobial properties has increased. In medicine, extracts from plants and their secondary metabolites and plant extracts have been used for many years, but now by the development of organic chemistry, pharmacology and medicine, we can determine which biologically active substances produced by these plants are useful. Antimicrobial activity described selected groups of plant secondary metabolites, which potentially would allow their use as antimicrobial substances in medicine. These substances can be complementary to the basic medical treatment because their main advantage is the lower incidence of side effects. This paper presents an overview of research on the antimicrobial properties of alkaloids, coumarins, flavonoids, essential oils, phytosterols, and phenolic acids. Natural substances that inhibit the growth of microorganisms are becoming an alternative to synthetic compounds, as confirmed by this literature review.

S t r e s z c z e n i e

C e l e m b a d a n i a jest odnalezienie substancji mogących wpływać na aktywność ruchową nabłonka w modelu eksperymentalnym poruszającego się ślimaka Achatina achatina. Ze względu na fizjologiczne, biochemiczne i fizyczne podobieństwa pomiędzy nabłonkami różnych gatunków, wyniki mogą mieć znaczenie dla mechanizmów fizjologicznego oddziaływania tych substancji.

M a t e r i a ł i m e t o d a. Sfilmowano proces ruchu ślimaków Achatina achatina zarówno spontaniczny jak i po wstrzyknięciu w stopę wybranych wcześniej neuroprzekaźników (serotoniny, kompleksu serotoninowo-kreatyninowego adrenaliny, noradrenaliny i dopaminy) oraz ambroksolu. Parametry mierzono wykorzystując filmy nagrane od spodu przez szklaną płytę po której poruszały się zwierzęta. Fale aktywności ruchowej nabłonka występowały na powierzchni stopy podczas ruchu.

W y n i k i i d y s k u s j a. Każda z badanych substancji miała wpływ na ruch ślimaków. Wstrzyknięcie serotoniny, kompleksu serotoninowo-kreatyninowego, noradrenaliny i ambroksolu spowodowało wzmożenie aktywności ruchowej ślimaków, z kolei adrenalina i dopamina nie powodowały takich zmian, lub też działały hamująco. Ważne parametry, których zmiany zaobserwowano, to długość fali skurczu i odległości między nimi, częstotliwość fali skurczu, prędkość ślimaka, przesunięcie ciała na jedną falę skurczu i wydajność skurczu.

W n i o s k i. Wyniki wskazują na możliwość zastosowania ślimaka A. achatina jako biologicznego modelu, ponieważ w czasie ruchu ślimaka można zaobserwować aktywność motoryczną nabłonka sterowaną przez układ nerwowy i wywoływaną skurczami tkanki mięśniowej, podobnie jak w ludzkich tkankach. Wstrzyknięcie serotoniny, kompleksu serotoninowo-kreatyninowego adrenaliny, noradrenaliny i dopaminy w organizmie modelowym A. achatina zmienia fale skurczu nabłonka na stopie. Postuluje się, że podobny proces zachodzi w innych nabłonkach, w tym ludzkich.

Key words: epithelium, Achatina achatina, motility, pedal wave

Słowa kluczowe: nabłonek, Achatina achatina, aktywność ruchowa, fale skurczu

INTRODUCTION

While general functions and physiology of epithelial tissue have been thoroughly recognized, less attention is focused on its motility, particularly the one related to movements of different substrates on epithelial surface. Various types of epithelium show different patterns of movement, which serves specific purposes, but the nature and exact mechanisms of its regulation are not fully known. To better understand the nature of these movements, a biological model could be helpful, aside from traditional methods which, like the Ussing apparatus, involve using tissue samples. In search for such a model, a set of similarities between snail pedal epithelium and other epithelia has been evaluated. As the animal moves forward series of repeated epithelial darkening areas called pedal waves are observed, which origin at the back of the foot and move forward towards the head as the animal moves forward [1]. Because snail movements are similar in physical nature to bowel movements, snails have the potential to be used as biological models. The wave progression and how it relates to overall mollusk adhesive locomotion in normal, unmodified conditions has already been studied [2,3,4]. The results showed that both pedal wave frequency and pedal wave length could play a key role in regulation of locomotion; however, these studies have so far proved inconclusive [5].

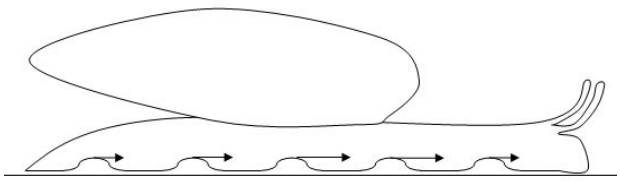

Fig. 1. *Side view of a moving snail illustrating the pedal waves where the epithelium tissue is lifted and contracted. Different vector lengths illustrate the variety of individual waves*

Ryc. 1. *Widok z boku na poruszającego się ślimaka ilustrujący fale na stopie, gdzie nabłonek ulega skurczeniu i podniesieniu. Różnice w wektorach ilustrują zróżnicowanie poszczególnych fal*

Another important factor is a thin layer of mucus produced by snail, on which it slides, and which alternately serves as adhesive material. Probably, the pedal waves are the way to regulate friction between the sole of the foot and the surface. The mucus, being a non-newtonian fluid, is efficient in transferring movement energy from the sole of the foot to the surface [6, 7, 8]. By its prosperities, the animal is also able to adapt to various conditions and use it more efficiently [9]. Mammalian epithelia, such as these covering the airways or gastrointernal tract, are also known to produce mucus that plays a key role in moving foreign substrates on their surfaces. In this study, the problem whether snails respond to pharmacological modification of their movement pattern was examined. Vulnerability to neurotransmitters, drugs and other chemical substances are a key aspect in considering the use of snails as a potential biological model of epithelium movement.

MATERIAL AND METHODS

The study used control and study groups of 10 Achatina achatina snails between 16 and 23 grams of body mass and 40 to 50mm of shell length. The snails were kept in a moist terrarium with a peat surface, at room temperature, and were fed with cabbage with addition of egg shells. During the study the snails were placed on a transparent glass surface, and their movement was then recorded with a digital camera CCD DFK 41 AV02.AS with a CCTV 5-50 mm F/1.8 lens, using IC Capture.AS 2.0 software. The recordings were then processed and examined using VirtualDub 1.9.9 and image analysis software. Recordings were all conducted starting at 9 a.m., at room temperature, and were approximately 30 seconds long per animal.

The following variables were used in the study: length of single pedal waves [mm], length of single intervals between pedal waves [mm], speed of snail's head [mm/s], frequency of pedal waves [1/s], total distance covered per pedal wave [mm] and epithelium fold coefficient. The last value was calculated by the following equation and shows how the distance covered by the animal corresponding with the pedal wave length:

$$u = \frac{shw}{shw+lw} \times 100\%$$

u – epithelium fold coefficient [%]
shw – distance covered per pedal wave [mm]
lw – single pedal wave length [mm]

During the study the following neurotransmitters were tested: epinephrine, norepinephrine, dopamine, serotonin (both pure and in a serotonin-creatinine complex). Additionally, ambroxol – a secretolytic agent and an inhibitor of Na+ that decreases mucus

density – was used to check the role of mucus. The studied neurotransmitters and ambroxol were applied in a form of an injection, dissolved in a physiological equivalent of Ringer's solution set specifically for the snails (80mmol/l NaCl, 4mmol/l KCl, 8mmol/l CaCl2, 5mmol/l MgCl2, 5mmol/l Tris, pH 7.8) [10]. The same solution was used in negative control groups. In each case the substance's dosage was 2 μg per gram of body mass. All variables were measured in a zero and negative control group and test groups of the same size (10 animals). The recordings were taken directly after drug admission and also an hour and two hours afterwards.

All the measured and calculated variables were then used to determine if the pharmacological modification of movement shows statistically significant results and wheatear these results can be applied to previously known data. To determine this, Welch's t test was used, with statistical significance at 0.05.

RESULTS

Serotonin in pure form had a statistically significant influence on variables describing motility. Directly after injection 82.05% increase in the animals' movement speed and 35.13 % in the value of epithelium fold coefficient was observed. These changes remained statistically significant up to an hour after admission. In comparison, the same serotonin in a creatinine complex did not cause a statistically significant change in movement speed, but affected the pedal wave generation, increasing their length by 38.34% and their frequency by 19.63%.

Epinephrine also did not show influence on the animals' movement speed, causing changes only in pedal wave generation. Wave length increased by 19.66% and the interval between waves increased by 19.28%, which in turn resulted in lesser epithelium contraction levels. On the other hand, norepinephrine caused a different set of changes, resulting in a major increase of 66.39% in movement speed, followed by a 24.65% increase in wave length.

Table I. *Movement variables after substance injection compared to control*

Tabela I. *Paramerty ruchu po wstrzyknięciu substancji w porównaniu z kontrolą*

Substance (substancja)	Single pedal wave length (długość fali skurczu) [mm]	Pedal wave interval length (odległość między falami) [mm]	Pedal wave to area compared total foot area ratio (stosunek powierzchni fali do całej stopy) [%]	Snail speed (prędkość ślimaka) [mm/s]	Pedal wave frequency (częstotliwość fali skurczu) [Hz]	Distance covered by the snail per pedal wave (odległość przebyta przez ślimaka podczas 1 fali) [mm]	Epithelium fold coefficient (współczynnik sfałdowania nabłonka) [%]
Serotonin (serotonina)	14,10% ±0,25 **	1,35% ±0,27	0,99% ±0,21	82,05% ±1,74	2,39% ±0,31	72,40% ±1,13	35,13% ±0,68
Serotonin complex (serotonina kompleks)	38,34% ±0,18	-6,56% ±0,21	-2,76% ±0,39	9,55%* ±0,64	19,63% ±0,16	69,28% ±0,3	14,95% ±0,14
Epinephrine (adrenalina)	19,66% ±0,39	19,28% ±0,48	17,65% ±0,41	-5,69% ±0,56	4,01% ±0,33	-10,00% ±0,5	-18,39% ±0,39
Norepinephrine (noradrenalina)	24,65% ±0,43	-7,69% ±0,29	30,57% ±0,47	66,39% ±0,39	10,60% ±0,32	45,54% ±0,42	12,16% ±0,32
Dopamine (dopamina)	6,77% ±0,11	-15,29% ±0,18	29,16% ±0,24	-3,48% ±0,62	-7,23% ±0,21	13,68% ±0,56	4,69% ±0,34
Ambroxol (ambroksol)	45,44% ±0,28	2,34% ±0,29	30,70% ±0,19	43,51% ±0,43	16,88% ±0,1	22,19% ±0,28	-12,01% ±0,13

* the increase in speed during the next hour was statistically significant. (w następnej godzinie zmiana była statystycznie istotna)

** results at p=0.05 (wyniki dla poziomu istotności p=0,05)

Values described as % difference according to control directly after neurotransmitter/amborxol admission. Numbers represent mean values and standard deviation. Statistically significant values are placed on gray background. (Wartości przedstawiono jako % różnicę w stosunku do kontroli bezpośrednio po podaniu neuroprzekaźników/ambroksolu. Wartości reprezentują średnie plus odchylenia standardowe. Wyniki istotne statystycznie przedstawiono na szarym tle.)

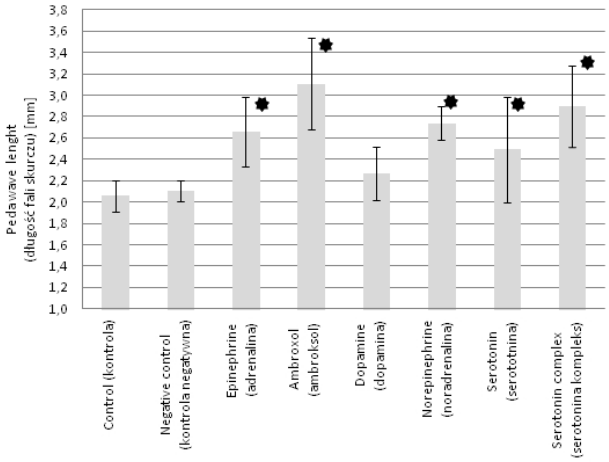

Fig. 2. *The effects of neurotransmitters and ambroxol on A. achatina pedal wave length directly after admission. The values shown are mean including standard deviation. Statistically significant results at p=0.05 are marked with **

Ryc. 2. *Efekt oddziaływania neuroprzekaźników i ambroksolu na długość fali skurczu u A. achatina bezpośrednio po podaniu. Pokazane wartości to średnie plus odchylenie standardowe. Istotne statystycznie wartości oznaczono **

The dopamine was used in this test as a reference to the previous studies. The only statistically significant changes observed were a 15.29% reduction in pedal wave interval length and a 29,15% increase in the folded area/total foot area ratio. Other motility variables remained below statistically significant levels.

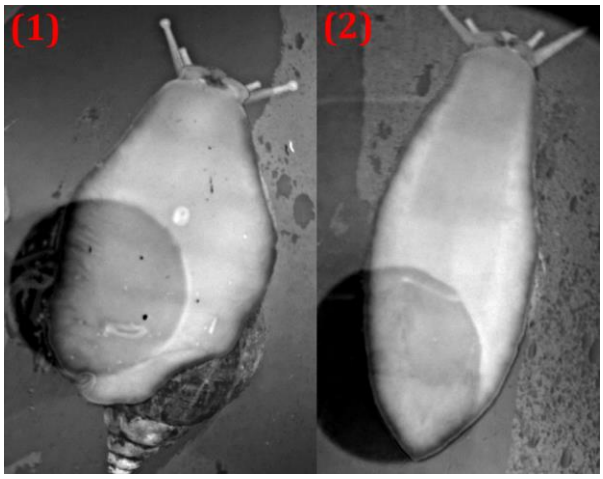

Fig. 3. *Sole of the snail foot with visible pedal waves (darker areas). 1 – spontaneous movement, 2 – directly after abroxol injection. The wave length and intervals between waves have increased*

Ryc. 3. *Stopa ślimaka z widocznymi falami skurczu [ciemne rejony]. 1 – ruch spontaniczny, 2 – bezpośrednio po podaniu ambroksolu. Długość fali i odległości pomiędzy nimi uległy zwiększeniu*

After the injection of ambroxol, a variety of changes in the movement pattern could be observed. The speed of the animals increased by 43,51%, with both pedal wave length and frequency increasing.

DISCUSSION

The results show a possibility for the use of A. achatina snail as a study model to test if the substances influence epithelial motility. The adhesive locomotion process responded to the pharmacological modification and thus, leads to believe that snails use an analogous set of receptors and neurotransmitters as mammals. The specific set of changes in movement speed and other variables differs according to the substance used, but follows a unique and characteristic pattern.

Being able to use the proposed model efficiently depends on the specific types of receptors and ion channels in the snail's body. The key to the further studies is to pinpoint their location and specific subtypes as this enables to fully evaluate the model. The proposed method is precise and repeatable enough to be considered for practical use. Also, the easiness of the process and animal breeding speed give chances for constituting a cheap and numerous research groups.

Finally, the role of mucus and its physical prosperities in the locomotors activity is not to be excluded. It has been proven before that mucus plays a key role in snail movement, and here it was shown that its modification drastically changes the movement pattern. A question of interest is if, and to what extent, such changes can be applied to model epithelium i.e. to modify the adhesive locomotion.

CONCLUSIONS

1. Serotonin, serotonin-creatinin complex, norepineprhine and ambroxol injected into snail's body cause an overall increase in movement speed while epinephrine and dopamine have no effect or decrease movement speed.
2. The increase in movement speed depends on the increase of pedal wave length, total distance covered per pedal wave, the pedal wave frequency and the epithelium fold percentage. Decrease in movement speed does not cause such changes or is followed by a decrease of those parameters.
3. It is proposed that in the process of adhesive locomotion of land snails an important role is played by epithelial motility regulated by the nervous system and caused by muscle contraction.
4. The above mentioned snail foot activities are a valuable biological model for testing if a substance influences the epithelial motility.

REFERENCES

1. Barker G. M.:. *The biology of terrestrial mollusks*, CABI Publishing, New Zeland 2001
2. Lissmann H.W.: *The mechanism of locomotion in gastropod mollusks I. Kinematics*, J. Exp. Biol., 1945, 21, 58-69
3. Lissmann H.W.: *The mechanism of locomotion in gastropod mollusks II. Kinetics*, J. Exp. Biol., 1945, 21, 37-50,
4. Tyrakowski T., Kaczorowski P., Pawłowicz W., Ziółkowski M.:, *Discrete movements of foot epithelium during adhesive locomotion of land snail*, Folia Biol. (Krakow) 2012, 60, 99-106

5. Lai J.H., del Alamo J.C, Rodriguez-Rodriguez J.: *The mechanics of the adhesive locomotion of terrestrial gastropods.* J. Exp. Biol., 2010, 213, 3920-3933
6. Denny M. W.: *A Quantitive Model for the Adhesive Locomotion of The Terrestial Slug, Ariolimax Columbians*, J. Exp. Biol., 1981, 91, 195-217
7. Denny M. W.: *Mechanical Prosperities of Pedal Mucus and Their Consequences for Gastropod Structure and Performance*, Amer. Zool., 1984, 24, 23-36
8. Denny M. W.: *The Physical Prosperities of the Pedal Mucus of the Terrestrial Slug Ariolimax columbianus*, J. Exp. Biol., 1980, 88, 375-393
9. Lauga E., Hosoi A.E.: *Tuning gastropod locomotion: Modeling the influence of mucus rhelology on the cost of crawling*, Phys. Fluids, 2006, 18:113102
10. Pavlova G.A.: *Effects of serotonin, dopamine and ergometrine on locomotion in the pulmonate mollusk Helix lucorum*, J. Exp. Biol., 2001 204, 1625-1633

Address for correspondence:
e-mail: wojciech-pawlowicz@wp.pl,
tomtyr@cm.umk.pl
Phone:
Author (+48) 664-194-557
Department (+48) 52-585-35-98

Received: 25.07.2012
Accepted for publication: 18.09.2012