

## The Effect of Serotonin on Epinephrine Modulation of Transepithelial Ion Transport in Isolated Frog Skin

Danuta Izabela KOSIK-BOGACKA and Tomasz TYRAKOWSKI

Accepted June 20, 2006

KOSIK-BOGACKA D. I., TYRAKOWSKI T. 2006. The effect of serotonin on epinephrine modulation of transepithelial ion transport in isolated frog skin. *Folia biol. (Kraków)* 54: 109-115.

The aim of this study was to examine the effect of serotonin and epinephrine on ion transport of isolated frog skin. The addition of serotonin after incubation in Ringer solution (RH), bumetanide (BUME), and after initial incubation in amiloride and subsequently in RH, reduced hyperpolarization and did not effect the mechanosensitivity of frog skin. Following incubation of the frog skin with amiloride (AMI), serotonin did not affect the value of hyperpolarization and increased mechanosensitivity. The addition of epinephrine (EPI) on frog skin incubated in RH and AMI did not affect hyperpolarization, but repeated application of this compound after serotonin increased hyperpolarization. After incubation with bumetanide, addition of EPI before and after application of serotonin did not affect the value of the examined parameters of the frog skin. Initial incubation with AMI and later in RH caused a drop in reaction to EPI and no effect on mechanosensitivity. Repeated addition of epinephrine in this group did not affect the reaction value, while it decreased the reaction value during mechanical stimulation. The experimental data presented in this study indicate that serotonin inhibits the sodium ion current. Epinephrine inhibits the chloride ion current, however, after the application of serotonin, EPI stimulates sodium ion transport.

Key words: Epinephrine, serotonin, frog skin, ion transport, Ussing apparatus.

Danuta KOSIK-BOGACKA, Chair and Department of Biology and Medical Parasitology, Pomeranian Medical University, Powstańców Wielkopolskich 72, 70-111 Szczecin, Poland.

E-mail: kodan@sci.pam.szczecin.pl

Tomasz TYRAKOWSKI, Department of Pathobiochemistry and Clinical Chemistry Medical University in Bydgoszcz, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland.

E-mail: tomtyr@aci.amb.bydgoszcz.pl

### Abbreviations:

AMI – amiloride

BUME – bumetanide

CGRP – calcitonin gene-related peptide

dPD – (in mV) is the difference between the maximum stimulation value of the transepithelial electrical potential difference

EPI – epinephrine

NANC – non adrenergic, non cholinergic

NKA – neurokinin A

PD – transepithelial electrical potential difference

RH – Ringer solution

SP – substance P

5-HT – serotonin

The transepithelial processes of absorption and secretion of ions in frog skin create and maintain a difference in the transepithelial electrical potential difference (PD), which is an electrophysiological exponent of ionic current in this organ. The value of this parameter is a combination of two processes (KOSIK-BOGACKA & TYRAKOWSKI 2001, 2002, 2003; TYRAKOWSKI *et al.* 1998 a, b). The first, relatively stable in time, is recorded as a stable transepithelial electrical potential difference (PD). The second is dependent on excitation by mechanical stimulation of nervous receptors on frog skin, which results in reversible and short-lived hyperpolarization (dPD). The processes of ion transport in the frog skin are beyond the control of the hormonal-, immune- and nervous systems.

The frog skin is richly innervated by the intradermal nervous system, forming a part of the autonomous system (KATZ & NAGEL 1994). Nerve fibres

of the frog skin can be excited both by mechanical (CALOF *et al.* 1981; CATTON 1976) and chemical stimuli (RANG *et al.* 1991), e.g. 5-HT. Based on the data, excitation of free nerve endings results in the secretion of neurotransmitters of the NANC system (NKA, SP, CGRP, and other), which modify ion transport through epithelia (BEVINS & ZASLOFF 1990; LIPPE *et al.* 1994).

The aim of this study was to examine the effect of serotonin and epinephrine on ion transport of isolated frog skin.

The objective of this study was to examine the effect of epinephrine on the value of the transepithelial electrical potential difference and mechanosensitivity of isolated frog skin before and after serotonin application.

## Material and Methods

### Animals

The experiments were carried out on male and female frogs (*Rana esculenta* L.), which were kept at 4°C. The studies were performed during the hibernation period of frogs (November till February). The experiment had been previously approved by the local Universities Committee for Ethical Animal Experiments.

### Experimental procedure

Experiments consisted of measurements of the transepithelial electrical potential difference (PD) of the skin placed in an Ussing apparatus. The Ussing apparatus had been modified as described previously (KOSIK-BOGACKA & TYRAKOWSKI 2001, 2002, 2003; TYRAKOWSKI *et al.* 1998 a, b). The principal modifications were as follows: 1) the tissue was mounted horizontally, 2) the nozzle, connected to a peristaltic pump by tubing, was fixed to the wall of the chamber in such a way that the jet flux of stimulation fluid could rinse the mucosal surface of the mounted tissue. PD was established when compensation current intensity of the external battery was  $I = \pm 0$  mA. The measuring equipment were a voltage/current clamp apparatus - EVC 4000 (WPI, USA) and a recorder - BD 111 (Kipp and Zonnen, Netherlands), which were connected to the Ussing apparatus by Ag/AgCl electrodes and agar bridges. Electrical stability tests of the experimental system (blank tests) were performed through application of the drugs on a synthetic cellophane membrane placed in the Ussing apparatus instead of living tissue.

### Isolation of frog skin

The frogs were stunned, decapitated and doubly pithed. Abdominal skin was carefully excised, divided into fragments about 2 cm<sup>2</sup>, and incubated in a well-aerated solution before mounting in the Ussing apparatus.

### Stimulation

Mechanical stimulation was performed by directing a jet of bathing medium on the mucosal surface of the skin. The pulsation of the rinsing fluid flow was maintained by a peristaltic pump; the fluid flowed from a nozzle mounted in the measuring chamber. The internal diameter of the nozzle was approximately 1.5 mm. It was placed at a distance of 12 mm from the mucosal surface of the tissue. Standard stimulation consisted of 8-9 jets of fluid, with a total volume of 2.50 ml applied over 30 s. The dPD which denotes the hyperpolarization during mechanical stimulation was calculated as the difference between the maximum stimulation effect and the control value PD before stimulation.

Combined mechanical and chemical stimulation were performed by the application of serotonin (0.005 mM, H-9523) and epinephrine (0.01 mM) on the mucosal surface of the skin.

### Solutions

The solutions used throughout the experiment were as follows – (concentrations in mM):

- Ringer solution of pH 7.4 (Na<sup>+</sup> 147.2, K<sup>+</sup> 4.0, Ca<sup>2+</sup> 4.4, Cl<sup>-</sup> 156, HEPES -N-2-hydroxyethyl-piperazine-N,-2-ethanesulfonic acid 10.0) – control group,

- Ringer solution with amiloride (0.1) – model of inhibited absorption of Na<sup>+</sup>,

- Ringer solution with amiloride, afterwards in Ringer solution – model of sodium ion transport unblocking,

- Ringer solution with bumetanide (0.1) – model of inhibited absorption of Cl<sup>-</sup>.

All chemicals were supplied by Simga-Aldrich Ltd., Poland.

### Statistical analysis

Results are given as a mean standard error of the mean (SEM). Statistical evaluation was made by the Student's *t*-test in the "Statgraphics" computer program. Differences were considered significant at the level of 0.05.

**Results**

Effect of 5-HT

The different incubation environments described above were used to test the effect of serotonin dPD and mechanosensitivity of frog skin (Table 1).

The addition of serotonin on frog skin incubated in Ringer solution lowered resulted in the disappearance of hyperpolarization by 46% in comparison with the mechanical stimulation and did not affect the mechanical stimulation after application of 5-HT (Table 1, Fig. 1a).

Incubation of the frog skin in amiloride eliminated the reaction after mechanical stimulation. The addition of 5-HT did not affect hyperpolarization and induced a reaction during mechanical stimulation after 5-HT of  $-1.0 \pm 0.4$  mV (Table 1, Fig. 1b).

The addition of serotonin after initial incubation of the skin with amiloride and subsequently in Ringer solution decreased hyperpolarization by 24% in comparison with the control reaction (MS) and did not affect the mechanical stimulation after 5-HT (Table 1, Fig. 1c).

After incubation of the frog skin with bumetanide, hyperpolarization decreased by some 76% in relation to control stimulation. The application of serotonin decreased hyperpolarization by 67% in comparison with the control reaction (MS). The value of the reaction to mechanical stimulation after application of serotonin did not differ from the reaction value before this compound was administered (Table 1, Fig. 1d).

Effect of epinephrine

The direct effect of epinephrine on dPD was examined, similarly to serotonin, along with the reaction value during mechanical stimulation (Table 1). Epinephrine was administered twice, before and after serotonin.

The addition of epinephrine on frog skin incubated in RH did not affect hyperpolarization, which was comparable to the control reaction (Table 1, Fig. 1a). Repeated application of this compound after serotonin increased the hyperpolarization by 46% (Fig. 2a). In both cases EPI did not affect the reaction value during mechanical stimulation.

After incubation of the frog skin with amiloride, the addition of EPI did not affect the values of the examined parameters. The reaction value during EPI application and during mechanical stimula-

Table 1

The influence of serotonin (5-HT) and epinephrine (EPI) modulation of hyperpolarization (dPD in mV) of isolated frog skin after mechanical stimulation (MS) by gentle, pulsatile rinsing

Experimental conditions (n)	MS	EPI	MS	5-HT	MS	EPI	MS
RH/RH (14)	-1.3±0.2 <sup>a</sup>	-1.2±0.2	-1.0±0.1	-0.7±0.1* <sup>a</sup>	-1.1±0.2	-1.9±0.3* <sup>a</sup>	-1.2±0.2
AMI/AMI (10)	0.0±0.1 <sup>b</sup>	-0.1±0.0	-0.1±0.0	-0.1±0.0	-1.0±0.4* <sup>b</sup>	-1.5±0.6* <sup>b</sup>	-0.9±0.3* <sup>b</sup>
AMI/AMI (RH) (10)	-2.1±0.2 <sup>c</sup>	-1.1±0.3* <sup>c</sup>	-1.8±0.5	-1.6±0.4	-1.9±0.5	-1.7±0.4	-1.3±0.3* <sup>c</sup>
BUME/BUME (10)	-0.3±0.0 <sup>d</sup>	-0.2±0.0	-0.2±0.0	-0.1±0.0* <sup>d</sup>	-0.2±0.0	-0.2±0.0	-0.2±0.0

The values represent the means ± SEM. The dPD (in mV) is the difference between maximum stimulation value of transepithelial electrical potential difference and that immediately before stimulation. The isolated skin was studied in an Ussing chamber and the solutions applied throughout the experiments were:

RH/RH – Ringer solution used as incubation, bathing and stimulation solutions;

AMI/AMI – Ringer solution with amiloride used as incubation, bathing and stimulation solutions;

AMI/AMI (RH) – incubation using Ringer solution with amiloride, afterwards Ringer solution used as incubation, bathing and stimulation solutions;

BUME/BUME – Ringer solution with bumetanide used as incubation, bathing and stimulation solutions;

MS – stimulation by pulsatile rinsing of the epithelial surface of skin by output from a peristaltic pump as described in experimental conditions;

EPI – epinephrine (0.01 mM) was added to the stimulation solution;

5-HT – serotonin (0.005 mM) was added to the stimulation solution.

\*a, \*b, \*c, \*d – significantly different from the a, b, c, d at P<0.05.

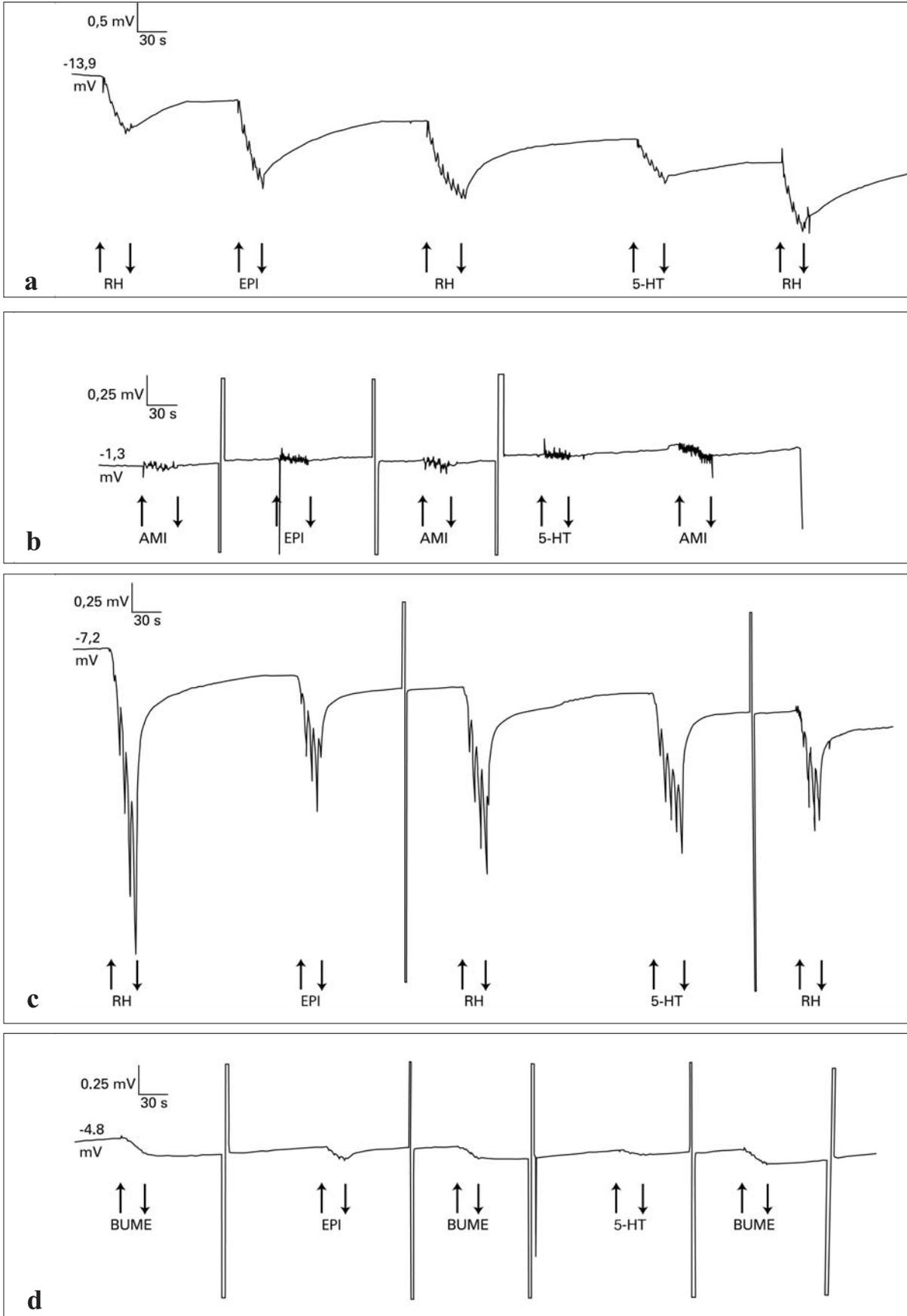


Fig. 1. The effects of epinephrine (EPI) and serotonin (5-HT) on the hyperpolarization of frog skin after mechanical stimulation (SM). A single experiment is shown. The tissues were incubated and bathed in the presence of Ringer solution only (a), or with the addition of amiloride (b), initially with amiloride, then in Ringer solution (c) and bumetanide (d). The arrows denote the beginning and end of the stimulus.

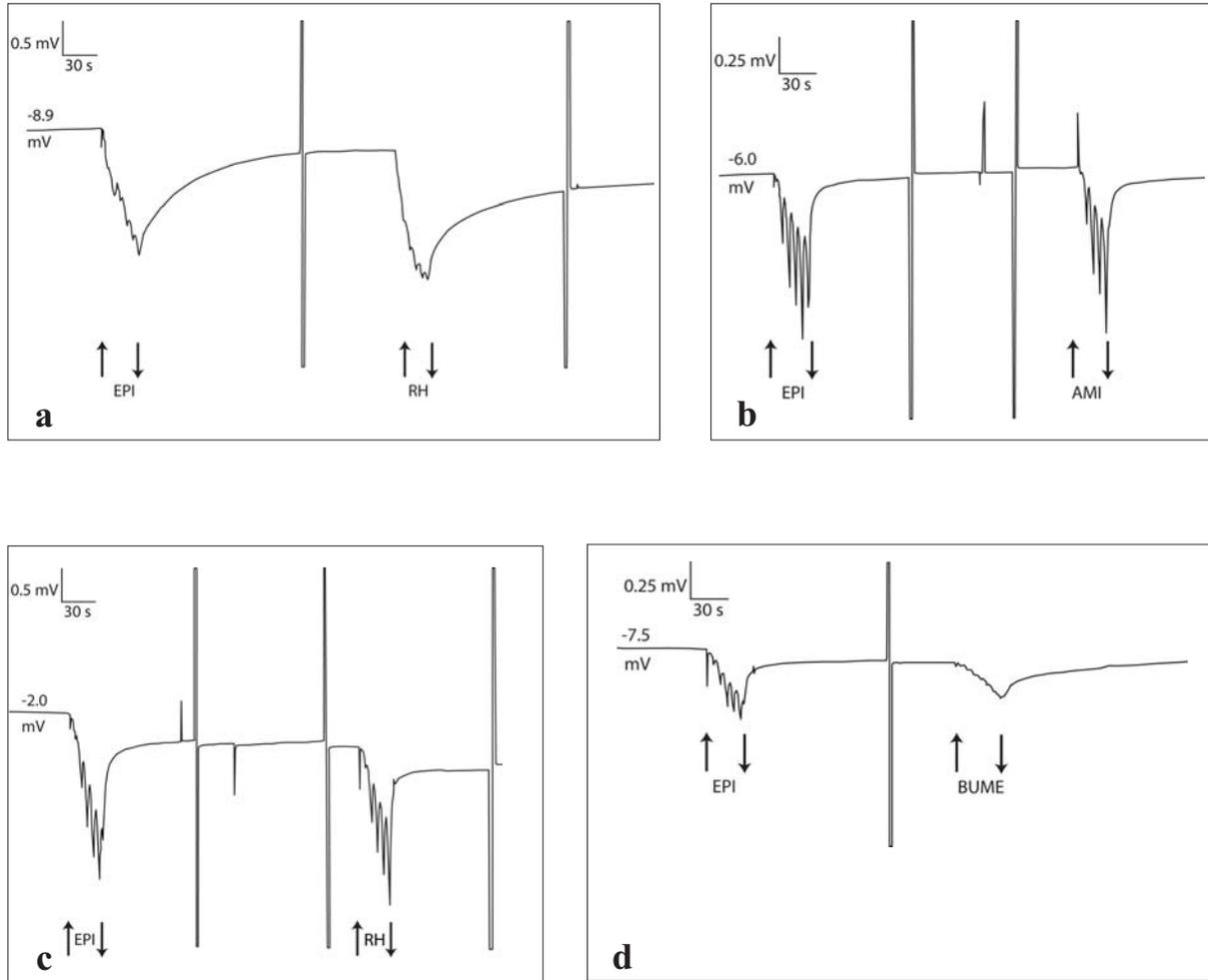


Fig. 2. The effect of repeated application of EPI after serotonin. A single experiment is shown. The isolated frog skin was incubated and bathed in the presence of Ringer solution only (a), or with the addition of amiloride (b), initially with amiloride, then in Ringer solution (c) and bumetanide (d). The arrows denote the beginning and end of the stimulus.

tion following the addition of this compound did not differ from the value of the control reaction (Table 1, Fig. 1b). Repeated application of EPI in this group resulted in hyperpolarization, the value of which was  $-1.5 \pm 0.6$  mV (Fig. 2b). Mechanical stimulation following the addition of EPI resulted in hyperpolarization, the value of which was  $-0.9 \pm 0.3$  mV.

The addition of epinephrine to frog skin initially incubated with AMI and subsequently in RH solution lowered the hyperpolarization by 48% in comparison with the control reaction and decreased the reaction value during mechanical stimulation by 14% (Table 1, Fig. 1c). Application of EPI after serotonin decreased by 19% the dPD and 38% the reaction value during mechanical stimulation (Fig. 2c).

After incubation with bumetanide, addition of EPI before and after application of serotonin did not affect the value of the examined parameters of the frog skin (Table 1, Fig. 1d and Fig. 2d).

## Discussion

### Basic electrophysiological parameters

Isolated frog skin is a basic model for electrophysiological tests using Ussing's chamber (KOEFOED-JOHNSEN & USSING 1958). It has been determined that in a physiological environment, this organ is in release state or is activated. The release condition reflects the constant transepithelial electrical potential difference (PD) which is created and modified by the local, transepithelial ion transport. The activated state is characterised by hyperpolarization (dPD). dPD depends on the stimulation of C-fibres and on the release of non adrenergic non cholinergic (NANC) neuropeptides, which stimulate electrogenic ion transport (KOSIK-BOGACKA & TYRAKOWSKI 2001, 2002, 2003).

On the basis of the experiment with inhibitors of the apical sodium channel (amiloride) and chlo-

ride channel (bumetanide) and literature on the subject (KOSIK-BOGACKA & TYRAKOWSKI 2001, 2002, 2003; USSING & ZERAHN 1951), it can be stated that the value of transepithelial electrical potential difference (PD) and dPD of the frog skin depend primarily on the transepithelial sodium transport, whereas the transport of chloride and other ions is less significant (Table 1).

#### Effect of 5-HT

5-hydroxytryptamine (5-HT, serotonin) is an important biogenic amine, which has effects on ion transport across the epithelium. Research on animals indicates that the effect of 5-HT on ion transport depends both on the animal species and organ.

In the renal tubules of dogs, 5-hydroxytryptamine stimulates active sodium transport (BLACKMORE 1958), in the trachea epithelium 5-HT stimulates chloride secretion and inhibits sodium absorption (TAMAOKI *et al.* 1997).

In the small intestine, 5-HT stimulates water and chloride secretion and inhibits NaCl absorption (HARDCASTLE *et al.* 1981; KISLOFF & MOORE 1976). A different effect of serotonin on transepithelial ion transport in different organs belonging to the same system may be related with differences in their innervation, corroborated by HARDCASTLE and HARDCASTLE (1997).

The effect of serotonin on frog skin depends on its concentration. The addition of 5-HT in low concentrations stimulates transepithelial sodium transport and reduces chloride transport (DALTON 1976). A high concentration of serotonin reduces chloride transport and inhibits sodium transport (DALTON 1977).

In the presented study, serotonin diminished the value of reaction after incubation in the control environment, after incubation with BUMETANIDE and after initial incubation with AMI and later in RH (Table 1).

These findings indicate that serotonin inhibits sodium transport, which shows that there is no effect of this compound on the reaction after inhibition of Na<sup>+</sup> transport (amiloride), and a decreased reaction was observed after an inhibitor of chloride transport (bumetanide) was administered.

#### Effect of epinephrine

The epithelium layer of the skin of *Rana esculenta* contains both  $\alpha$ - and  $\beta$ -adrenergic receptors (RAJERISON *et al.* 1972). A commonly described reaction of epinephrine to ionic transport was the intensification of chloride ion secretion (WELSH 1987).

Epinephrine increases the short circuit current in isolated frog skin (USSING & ZERAHN 1951). It has been found that the effect of norepinephrine on ion transport in the frog skin depended on the concentration of this hormone. A low concentration of norepinephrine increased the short circuit current by increasing the active influx of sodium ions (BASTIDE & JARD 1968). A high concentration increased the outflux of sodium ions and influx of chloride ions, which is probably related with the effect of norepinephrine on mucous glands (GUDME *et al.* 2000). WATLINGTON (1968, 1969) argued that the stimulation of receptors located in epithelial cells of frog skin reduced active sodium transport, whereas the stimulation of receptors occurring in mucous glands increased the influx of Na<sup>+</sup>.

In this study, epinephrine administered in control conditions after blocking apical sodium channels by amiloride, did not affect the magnitude of hyperpolarization and the reaction value during mechanical stimulation after the application AD (Table 1). The lack of reaction to AD in these groups results from the fact that hyperpolarization in the frog skin depends primarily on sodium transport, while chloride transport plays a less significant role. This corroborates the fact that there is a reduction in reaction to epinephrine after the application of the inhibitor of chloride transport (bumetanide) for incubation of the frog skin.

Repeated addition of this compound after 5-HT in control conditions and after blocking apical sodium channels resulted in an increase in the reaction values. The reaction value during mechanical stimulation in the RH group did not change, whereas it increased in the AMI group in comparison with the control reaction. This resulted from the stimulation by AD of sodium transport blocked by serotonin.

An entirely different effect was observed after administering EPI on the skin initially incubated with AMI and later in RH. Under these circumstances, epinephrine caused a drop in reaction and did not affect the reaction value during mechanical stimulation. This may be related to the inhibiting effect of epinephrine on the secretion of chlorides, since during rinsing of amiloride from tissues by Ringer solution, not only sodium but also chloride channels are stimulated. This hypothesis is corroborated by a high PD value in this group, even higher than in the controlled environment and a slight reduction in reaction to serotonin. This hypothesis is further corroborated by the reaction of this group to a repeated addition of EPI. The application of epinephrine did not affect the reaction value and reduced it during mechanical stimulation.

In conclusion, this study indicates that serotonin inhibits the sodium ion current. Epinephrine inhibits the chloride ion current, but after application of serotonin EPI stimulates sodium ion transport.

## References

- BASTIDE F., JARD S. 1968. Action of noradrenaline and oxytocin in the active transport of sodium and the permeability of frog skin to water. Role of cyclic 3', 5'-AMP. *Biochim. Biophys. Acta* **150**: 113-123.
- BEVINS C. L., ZASLOFF M. 1990. Peptides from frog skin. *Ann. Rev. Biochem.* **59**: 395-414.
- BLACKMORE W. P. 1958. Effect of serotonin on renal hemodynamics and sodium excretion in the dog. *Am. J. Physiol.* **193**: 639-643.
- CALOF A. L., JONES R. B., ROBERTS W. J. 1981. Sympathetic modulation of mechanoreceptor sensitivity in frog skin. *J. Physiol.* **310**: 481-499.
- CATTON W. T. 1976. Adaptation in mechanoreceptors of amphibian skin. *Prog. Brain Res.* **43**: 227-231.
- DAJERISON T. 1977. The effect of 5-hydroxytryptamine creatinine sulphate on sodium transport across isolated frog skin. *Comp. Biochem. Physiol.* **56**: 41-47.
- DALTON T. 1976. The loss of biological activity of 5-hydroxytryptamine creatinine sulphate. *Experientia* **32**: 1421-1423.
- GUDME C. N., NIELSEN M. S., NIELSEN R. 2000. Effect of alpha-adrenergic stimulation of Cl<sup>-</sup> secretion and signal transduction in exocrine glands (*Rana esculenta*). *Acta Physiol. Scand.* **169**: 173-182.
- HARDCASTLE J., HARDCASTLE P. T. 1997. Comparison of the intestinal secretory response to 5-hydroxytryptamine in the rat jejunum and ileum *in vitro*. *J. Pharm. Pharmacol.* **49**: 1126-1131.
- HARDCASTLE J., HARDCASTLE P. T., REDFERN J. S. 1981. Action of 5-hydroxytryptamine on intestinal ion transport in the rat. *J. Physiol.* **320**: 41-55.
- KATZ U., NAGEL W. 1994. Biophysics of ion transport across amphibian skin. (In: *Amphibian Biology, The Integument*, vol. 1, Heatwole, H., Barthalmus G. T. eds. Surrey Beaty and Sons PTY Ltd., 1994, Chipping Norton, Australia): 98-119.
- KISLOFF B., MOORE E. W. 1976. Effect of serotonin on water and electrolyte transport in the *in vivo* rabbit small intestine. *Gastroenterology* **71**: 1033-1038.
- KOEFOED-JOHNSEN V., USSING H. H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**: 298-308.
- KOSIK-BOGACKA D. I., TYRAKOWSKI T. 2001. Electrophysiological effect of ruthenium red and pH on the skin of the edible frog, *Rana esculenta* L. *Folia biol. (Kraków)* **49**: 273-277.
- KOSIK-BOGACKA D. I., TYRAKOWSKI T. 2002. Transepithelial transport of sodium and chloride ions in isolated skin of the frog, *Rana esculenta* L. *Folia biol. (Kraków)* **50**: 107-114.
- KOSIK-BOGACKA D. I., TYRAKOWSKI T. 2003. Effect of capsaicin and dimethyl sulphoxide (DMSO) on ion transport in isolated skin of frog (*Rana esculenta*). *Folia biol. (Kraków)* **51**: 117-123.
- LIPPE C., BELLANTUONO V., CASTRONUOVO G., ARDIZZONE C., CASSANO G. 1994. Action of capsaicin and related peptides on the ionic transport across the skin of *Rana esculenta*. *Arch. Int. Physiol. Biochim. Biophys.* **102**: 51-54.
- RAJERISON R. M., MONTEGUT M., JARD S., NOREL F. 1972. The isolated frog skin epithelium: presence of  $\alpha$  and  $\beta$  adrenergic receptors regulating active sodium transport and water permeability. *Pflügers Arch.* **332**: 313-331.
- RANG H. P., BEVAN S., DRAY A. 1991. Chemical activation of nociceptive peripheral neurones. *Brit. Med. Bull.* **47**: 534-548.
- TAMAOKI J., CHIYOTANI A., TAKEMURA H., KONNO K. 1997. 5-hydroxytryptamine inhibits Na absorption and stimulates Cl secretion across canine tracheal epithelial sheets. *Clin. exp. Allergy* **27**: 972-977.
- TYRAKOWSKI T., BANACH B., MOŚCIBRODA A., BARTŁOMOWICZ M., WOJCIECHOWSKA M. 1998. Reappraisal of amiloride action on transepithelial electrical potential difference of isolated tracheal wall. *Arch. Immunol. Therp. Exp. Warszawa* **46**: 45-50 a.
- TYRAKOWSKI T., BANACH B., GRECZKO I., BARTŁOMOWICZ M., WOJCIECHOWSKA M. 1998. Electrophysiological study of the interaction between epithelium and the airway fluid lining. *Int. Rev. Allergol. Clin. Immunol.* **4**: 59-65 b.
- USSING H. H., ZERAHN K. 1951. Active transport of sodium as source of electric current on the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**: 109-127.
- WATLINGTON C. O. 1968. Effect of catecholamines and adrenergic blockage on sodium transport of isolated frog skin. *Am. J. Physiol.* **214**: 1001-1010.
- WATLINGTON C. O. 1969. Alpha-adrenergic inhibition of Na<sup>+</sup> transport: the interaction of vasopressin and 3', 5'-AMP. *Biochim. Biophys. Acta* **193**: 394-402.
- WELSH M. J. 1987. Electrolyte transport by airway epithelia. *Physiol. Rev.* **67**: 1143-1184.