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EFFECT OF CAPSAICIN ON ION TRANSPORT IN THE CAECUM OF RABBITS

Danuta Kosik-Bogacka[1,#], Bolesław Banach[2], Tomasz Tyrakowski[3]

[1]Department of Biology and Medical Parasitology and [2]Department of Physiology, Pomeranian Medical University, Powstańców Wlkp. 72, PL 70-111 Szczecin, Poland, [3]Department of Electrophysiology of Epithelial Tissue, The Ludwik Rydygier Medical University in Bydgoszcz, M. Skłodowskiej-Curie 9, PL 85-094 Bydgoszcz, Poland

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Effect of capsaicin, a stimulator of C-fibres, on ion transport in the caecum of rabbits was studied using electrophysiological methods, designed to evaluate ionic currents occurring in epithelial tissues. The experiments consisted in measuring transepithelial electrical potential difference (dPD) of an isolated fragment of rabbit's caecum, placed in a Ussing apparatus. The ion transport was modified through incubation in Ringer solution, supplemented with amiloride, bumetanide, and capsaicin. Capsaicin was also administered with peristalting pump.

The experiments demonstrated that the inhibition of sodium ions transport caused by incubation with amiloride and incubation with capsaicin slowed down mechanical reaction to electrical potential difference. On the other hand, immediately after the administration, the capsaicin effect on C-fibres modified electrophysiological reaction of the caecum to mechanical stimulation. Physiological and pharmacological experiments reveal that a component dependent on activation of C-fibres contributes to the reaction of ion transport activation following mechanical stimulation.

Key words: *amiloride, bumetanide, caecum, capsaicin, ion transport, Ussing apparatus*

[#] *correspondence*; e-mail: kodan@sci.pam.szczecin.pl

INTRODUCTION

The principal component of the chilli pepper, capsaicin, is a representative of pharmaceuticals irritating sensory endings, C-fibres in particular, but it exerts irritating action also on other tissues, which is expressed by their congestion [5, 20, 21, 24]. It has also been demonstrated that the activation of C-fibre endings by capsaicin is related to the release of neuropeptides of the non-adrenergic, non-cholinergic (NANC) system [substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP)] to the surrounding tissues. The listed above neuropeptides affect the performance of smooth muscles of the digestive tract, mucus production by submucosal glands and transepithelial ion transport [7–9, 12, 19–22, 24, 26, 30, 38]. Release of neuropeptides of the NANC system can also lead to a neurogenic inflammation [3, 10, 27]. It leads to a local congestion, increased permeability of the blood vessels and epithelium, and to release of other typical inflammatory mediators. In addition, an excessive activation of C-fibres in the course of epithelial cell destruction causes antidromic activation of all dendrites stimulating NANC neuropeptide release (axonal reflex). In consequence, muscle contraction occurs along with activation of mucus secretion and ion transport stimulation [8, 9, 11, 15, 16, 24, 33].

In recent years, a number of works were published on ion transport in the intestine of mammals [11, 12, 16, 19–22, 32, 34]. Studies on ion transport in the large intestine of rabbits revealed that the principal factor contributing to the changes in the transepithelial electrical potential difference in this organ was chlorine ion transport [19–22].

The aim of this work was to determine capsaicin action on the electrical potential difference of an isolated wall of the rabbit caecum, before and after pharmacological modification of the ion transport by amiloride and bumetanide.

MATERIALS and METHODS

The experiments were carried out on 136 fragments of caecum collected from 20 not outbred rabbits of both sexes, weighing 3–4 kg, provided by the Animal Experimental Unit of the Pomeranian University in Szczecin.

The experiments consisted in measuring transepithelial electrical potential difference (dPD in mV) of an isolated intestinal wall, placed in an apparatus designed for studying electrical parameters of isolated epithelial tissues, a Ussing apparatus [18]. The Ussing apparatus was connected through Ag/AgCl electrodes and agar bridges filled with KCl solution, to EVC 4000 apparatus (manufactured by WPI, USA) and BD 111 recorder (manufactured by Kipp & Zonnen, The Netherlands). Effects of actions stimulating the measuring system (introduction of solutions) were verified in the presence of a cellophane barrier in the experimental chamber and they were treated as control.

The rabbits were killed by CO_2 asphyxiation and immediately operated. The caeca were cut out and their contents were removed through gentle rinsing. Then they were submerged in Ringer solution (36°C), cleaned of connective tissue, opened longitudinally and divided into pieces. The caecum fragments were incubated in an incubation fluid for about 1 h, and then placed in the apparatus. A piece of the caecum wall between tapes, measuring some 2 cm^2, constituted the experimental surface.

Mechanical stimulation was induced by a stream of the fluid, present in the chamber of Ussing apparatus, directed on the mucous surface of the caecum. Hyperpolarization reaction induced this way was referred to as dPD. The fluid stream was delivered by a nozzle, 1.5 mm in diameter, situated 12 mm from the studied organ. A standard stimulus lasted 30 s and it consisted of 8 squirts of the fluid, amounting to some 2.45 ml.

In addition to the mechanical stimulus, a chemical one was applied as well. It consisted of a gentle application of capsaicin, with the aid of a pipette on the mucous surface of the caecum.

The following incubation media were used also for filling up the chambers of Ussing apparatus at the time of the present experiment: Ringer solution (ionic composition in mM: Na^+ 147.2; K^+ 4.0; Ca^{2+} 4.4; Cl^- 155.6; HEPES 10.0) and Ringer solution supplemented with amiloride (0.1 mM), bumetanide (0.1 mM) with dimethyl sulfoxide, capsaicin (0.0001%) (all supplied by Sigma Chemical Co.). The solution of capsaicin at the concentration of 0.0001% was applied as a dose of 20 μl directly on the surface of the organ, serving as a test measurement.

Statistical hypotheses were verified using chi square test for assessment of qualitative data, while Student's *t*-test was used for evaluation of quantitative data. Significance limits were assumed at

$p < 0.05$. The calculations were carried out using "Statgraphics" computer software.

RESULTS

Table 1 contains information on the effect of different incubation conditions on PD and the reaction of an isolated wall of rabbit caecum to mechanical stimuli. Mechanical stimulation in a form of rinsing, applied on a caecum fragment, previously incubated in Ringer solution, caused transient increase in the electrical potential value (hyperpolarization), which was shown in Figure 1. The increase in electrical potential difference occurred immediately after the application of a mechanical stimulus (Fig. 1a). The highest hyperpolarization was observed usually before the end of a 30-s stimulation. When the stimulation was over, the value of electrical potential difference returned to control value within 5–10 min (Fig. 1a).

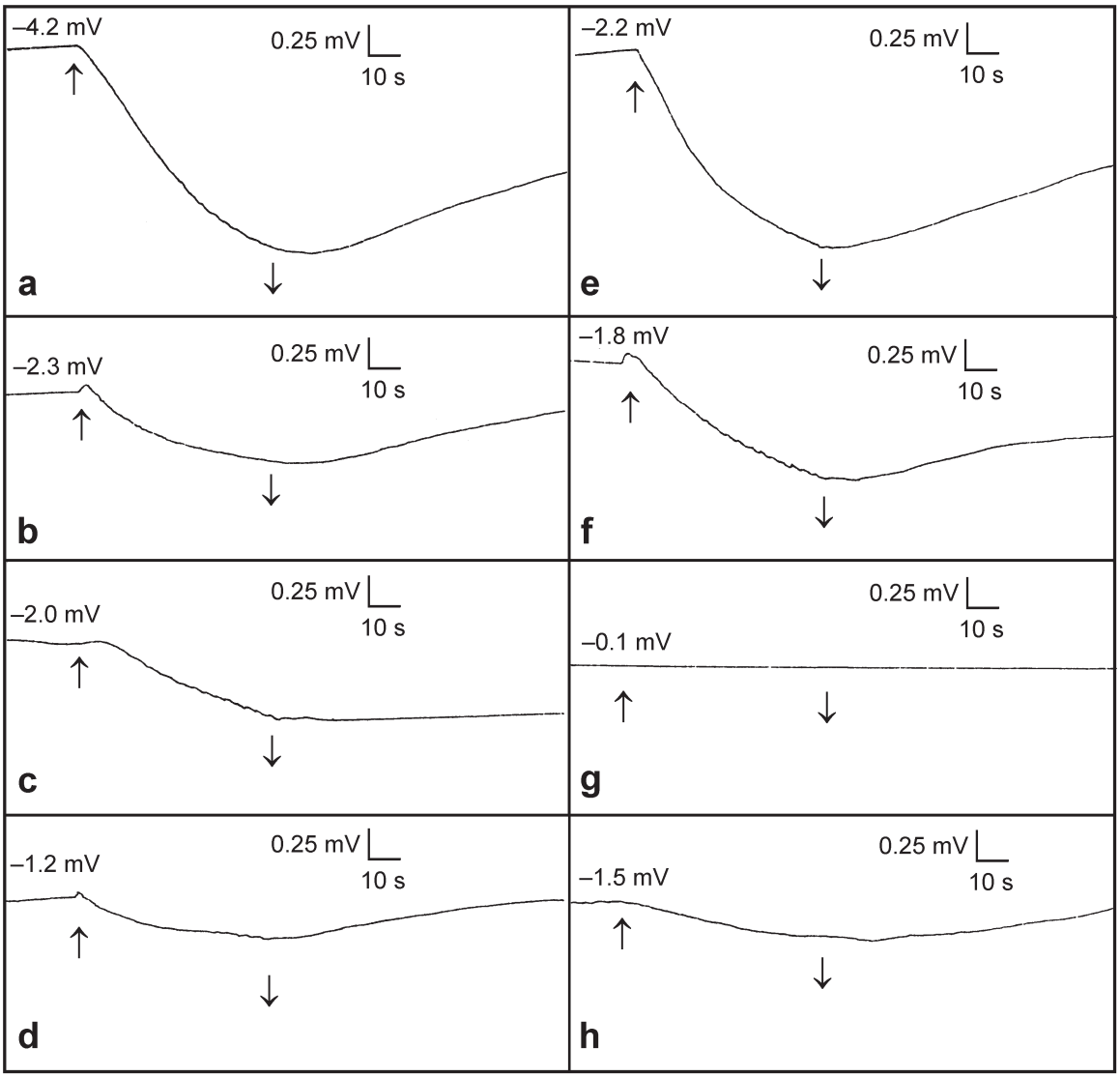

Fig. 1. Time-course of the changes in transepithelial potential difference (dPD) after mechanical stimulation of isolated rabbit intestine under different incubation conditions. The tissues were incubated in different solutions: **a** – Ringer solution, **b** – Ringer solution with capsaicin solvent, **c** – Ringer solution with capsaicin, **d** – first hour in Ringer solution with capsaicin and than one hour in Ringer solution alone, **e** – Ringer solution with amiloride, **f** –first hour in Ringer solution with amiloride and then one hour in Ringer solution alone, **g** – Ringer solution with bumetanide, **h** – first hour in Ringer solution with amiloride, bumetanide and then one hour in Ringer solution alone. Stimulation was 30 s of jet rising. The start and finish of stimulation are denoted by the pair of arrows. Single experiments are shown

Table 1. The electrophysiological variables of the isolated rabbit intestine wall

Type of incubation (n)	PD (mV)	MS [1] dPD (mV)
RH n = 20	–3.0 ± 0.4	–1.2 ± 0.2
AMI n = 15	–2.5 ± 0.4	–0.6 ± 0.2*
AMI/RH n = 14	–1.4 ± 0.2*	–1.0 ± 0.2
BUME n = 20	–0.9 ± 0.2*	–0.2 ± 0.1*
BUME/RH n = 7	–1.1 ± 0.5*	–0.5 ± 0.1*
CAPSA n = 17	–1.7 ± 0.3*	–0.7 ± 0.1*
CAPSA/RH n = 15	–0.7 ± 0.1*	–0.5 ± 0.1*

Rabbit caecum was incubated for 60 min in Ringer solution (**RH**) or in Ringer solution supplemented with amiloride (**AMI**), in initial phase in Ringer solution with AMI and later in Ringer solution alone (**AMI/RH**), bumetanide (**BUME**), in initial phase in Ringer solution with BUME and later in Ringer solution alone (**BUME/RH**), capsaicin (**CAPSA**), or, in initial phase in Ringer solution with CAPSA and later in Ringer solution alone (**CAPSA/RH**). Subsequently, after placing in Ussing apparatus, the rabbit inestine wall was flushed with stimulating fluid from the external surface (**MS**). [1] – dPD is the difference between the maximum stimulation value and the control value; n – number of preparations, * – significantly different from RH group at $p < 0.05$

Table 2. Effect of capsaicin (CAPSA) on the electrophysiological variables of the isolated rabbit intestine wall

Type of incubation (n)	MS1 dPD (mV)	CAPSA dPD (mV)	MS2 dPD (mV)
RH n = 20	–1.2 ± 0.2	–2.0 ± 0.3*	–1.8 ± 0.2*
AMI n = 15	–0.6 ± 0.2	–1.8 ± 0.3*	–0.9 ± 0.2
AMI/RH n = 14	–1.0 ± 0.2	–1.3 ± 0.3	–0.9 ± 0.2
BUME n = 20	–0.2 ± 0.1	–0.9 ± 0.2*	–0.4 ± 0.1
BUME/RH n = 7	–0.5 ± 0.1	–1.1 ± 0.2*	–0.4 ± 0.1
CAPSA n = 17	–0.7 ± 0.1	–0.8 ± 0.2	–0.7 ± 0.1
CAPSA/RH n = 15	–0.5 ± 0.1	–0.7 ± 0.1	–0.5 ± 0.1

Rabbit caecum was incubated for 60 min in Ringer solution (**RH**) or in Ringer solution supplemented with amiloride (**AMI**), in initial phase in Ringer solution with AMI and later in Ringer solution alone (**AMI/RH**), bumetanide (**BUME**), in initial phase in Ringer solution with BUME and later in Ringer solution alone (**BUME/RH**), capsaicin (**CAPSA**), or, in initial phase in Ringer solution with CAPSA and later in Ringer solution alone (**CAPSA/RH**). **MS1** – mechanical stmulation before administration of capsaicin and **MS2** – mechanical stmulation after administration of capsaicin, n – number of preparations, * – significantly different from MS1 of the same incubation group at $p < 0.05$

Incubation of rabbit caecum in the presence of amiloride did not affect the value of transepithelial electrical potential difference, but it lowered the reaction value after mechanical stimulation by some 50% (Fig. 1e). Similarly as in the case of control incubation, the value of electrical potential started to increase when the stimulus was applied. When the stimulation was over the potential remained at a stable level and it did not return to the initial level (Fig. 1e). Incubation of the caecum, initially with amiloride and later in Ringer solution caused a decrease in the transepithelial electrical potential by 60%. The reaction value after mechanical stimulation did not differ from the value of control stimulation (incubation in Ringer solution) (Fig. 1f).

After incubation of the caecum wall with bumetanide, the transepithelial electrical potential decreased by some 70% in relation to the control incubation. During mechanical stimulation (in the presence of bumetanide) the value of hyperpolarization decreased by some 80% in relation to the control stimulation (Fig. 1g). Removal of bumetanide, when initial bumetanide incubation was followed by incubation with Ringer solution caused an increase in reactivity of rabbit caecum (Fig. 1h), at the same time not affecting the value of electrical potential.

Introduction of capsaicin, a C-fibre stimulator, into incubation medium and stimulation of caecum walls caused a decrease in the electrical potential value and the reaction value after mechanical stimulation by some 40%. At the end of stimulation under the same experimental conditions, value of transepithelial electrical potential difference maintained itself at a stable level, and it did not go back to the initial value (Fig. 1c). Incubation with capsaicin, replaced later by Ringer solution, and incubation in capsaicin solvent lowered the value of electrical potential by some 75%, while the reaction after mechanical stimulation was reduced by some 55% (Fig. 1d and b).

Table 2 contains information about the effect of capsaicin on electrophysiological parameters of the rabbit caecum wall. Administration of capsaicin to the epithelium of caecum incubated in Ringer solution caused an increase in the reaction value by some 40% in relation to the control stimulation (Fig. 2a and c). The value of electrical potential started to increase immediately after application of the stimulus. It lasted about 10 s and then the value

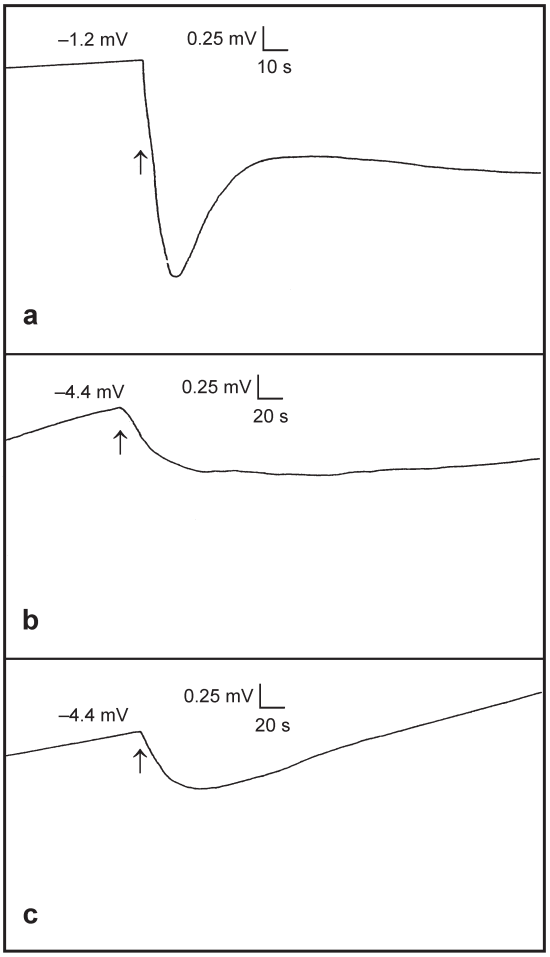

Fig. 2. Transepithelial electrical potential difference (dPD) of the isolated rabbit intestinal wall after chemical stimulations: **a** – with capsaicin, **b** – with capsaicin solvent (Tween 80), **c** – with Ringer solution. The tissues were incubated 1 hour in Ringer solution before stimulation. Single experiments are shown

of potential declined. Usually after 30–60 s from the moment of stimulus application, the electrical potential stabilized itself, but it did not go back to the initial value (Fig. 2a). Administration of capsaicin solvent on caecum incubated in Ringer solution caused different effect than capsaicin. Capsaicin solvent (Tween 80) caused also a hyperpolarization, but its value was lower by some 60% (Fig. 2b). Post-stimulation reaction values, before and after capsaicin administration, under the same experimental conditions, did not differ from each other.

Capsaicin administered on the caecum incubated with amiloride increased three times the reaction value in relation to the solvent administration. Reaction value to mechanical stimulation in this group was not affected by the administration of capsaicin. Caecum, initially incubated with amiloride and later with Ringer solution reacted with hyperpolarization to capsaicin supplementation. The hyperpolarization value did not differ from the control stimulation. Reaction value after the mechanical stimulation did not differ before and after the administration of capsaicin.

Capsaicin applied on epithelium of the caecum incubated with bumetanide caused a four-fold reaction increase, at the same time causing no changes in the reaction to mechanical stimulation. On the other hand, initial incubation with bumetanide followed by incubation with Ringer solution caused a two-fold increase in the reaction. Similarly as in the group incubated with bumetanide, the reaction value to mechanical stimulation did not differ before and after administration of capsaicin.

Capsaicin applied on epithelium of the rabbit caecum incubated initially with capsaicin, replaced later with Ringer solution, did not have any effect on the reaction value nor on the reaction value after mechanical stimulation.

DISCUSSION

The model of an isolated wall of mammalian intestine has been widely used for the studies on the role of ion transport in the course of different processes related to physiology as well as to pathophysiology of alimentary tract.

Our modification of a classical electrophysiological model consisted in addition of a nozzle linked to a peristaltic pump in a chamber of a Ussing apparatus [18]. Such modification enables irritation of sensory receptors on the studied organ, by a stream of a nourishing fluid rinsing the epithelium of rabbit caecum. It is evident from the literature data that such or similar actions stimulate sensory receptors, C-fibres in particular [5, 19–22, 24, 27, 28, 35–37].

The presently studied transepithelial electrical potential difference of a caecum is an important physiological parameter determining functional state of this organ occurring upon secretion of chloride ions and reabsorption of sodium ions [1, 6, 11, 13, 16, 19–23, 33, 34, 38]. This process leads to segregation of electrical charges, which demonstrates itself as building up negative charges in the mucus layer, covering epithelium of the caecum, and positive charges – in the submucosal layer [19–22].

The present work demonstrated that electrical potential, otherwise stable for long periods (hours, *in vitro* studies), can undergo transient, reversible changes in a form of hyperpolarization during action of mechanical stimuli (Tab. 1; Fig. 1a). The presently used stimulus is similar to a physiological situation. Rinsing the epithelium of rabbit caecum stimulates movement of mucus on the surface of epithelium. Each application of fluid by a peristaltic pump seems to be treated by the receptor system as an independent stimulus. These changes suggest an involvement of sensory receptors and lead to an assumption that at the time of stimulation, the sensory endings release NANC system neuromediators, which stimulate epithelial cells to change their electrogenic ion transport. A similar reaction to mechanical stimulation has been described for rabbit trachea [19, 21, 35, 36] and also for frog skin [19, 20, 37].

Based on the presently described experiments and the literature data, it can be assumed that hyperpolarization following mechanical stimulation is not only a direct reaction of ion channels sensitive to stimuli and present in the endings of afferent (sensory) fibres, but it contributes to a multi-link regulatory system. In view of publications describing the role of afferent fibres of autonomic system (also vagus nerve) and neuropeptides of the NANC system [17, 24], it is possible that afferent endings of C-fibres [19–22, 28, 35–37] (reacting to fluid movement on the surface of epithelium) are elements sensitive to mechanical stimuli. On the other hand, the systems of transepithelial transport of sodium and chloride ions in epithelial cells are response elements. Molecules of NANC system neuropeptides secreted from C-fibre endings and subsequently diffusing into the intercellular space form a link between both elements.

Two ion transport inhibitors: amiloride and bumetanide were used to determine the pathway or pathways of the ion transport responsible for the changes in the electrical potential difference. Amiloride is a known and commonly used blocker of the sodium channel [2, 4, 14, 19–22, 29, 31, 35–37]. In this study, amiloride was used for both incubation and rinsing of the caecum. In the presence of amiloride in the organ studied and in nourishing and stimulating fluid, the overall picture of electrical phenomena depends on the pathway of chloride ion transport. An attempt to block the reaction to mechanical stimulation through blocking sodium channels using amiloride has failed. Such experimental conditions caused only a decline in transepithelial electrical potential difference by some 50%, but they did not eliminate entirely the hyperpolarization PD after stimulation with mechanical stimuli (Tab. 1; Fig. 1e). It is possible that the blockage of ion channels by amiloride was relatively persistent, because removal of amiloride by subsequent incubation in Ringer solution did not change reaction of rabbit caecum to mechanical stimulation (Tab. 1; Fig. 1f). In addition to amiloride, also effect of bumetanide on ion transport was studied experimentally on an isolated rabbit caecum. Bumetanide has been known as an inhibitor of transepithelial transport of chlorine ions, through blockage of basolateral co-transport mechanism $Na^+K^+2Cl^-$ [19–22, 25, 37]. In the presence of bumetanide in the incubation and stimulating medium, the dPD reaction depends solely on sodium ion transport. Such experimental conditions can be described as a pharmacological isolation of sodium current. The attempt to influence electrophysiological parameters of an isolated rabbit caecum through blockage of chloride transport by bumetanide caused a decrease in electrical potential by some 90%. It also contributed to a decrease in hyperpolarization value after mechanical stimulation (Tab. 1; Fig. 1g). Similarly as it was in the case of amiloride, the reaction of caecum to bumetanide was relatively persistent. Initially, the incubation with bumetanide, followed by incubation in Ringer solution did not affect the value of reaction to mechanical stimulation (Tab. 1; Fig. 1h). These experimental data indicate that the reaction of rabbit caecum after mechanical stimulation depends primarily on opening of apical ion channels for chlorides and sodium ions, while the channels for other ions play a less important role.

Rabbit caecum is a richly innervated organ, containing, among others, sensory receptors. It has been demonstrated in the literature that C-fibre stimulation did not only induce afferent impulses, but also triggered secretion of neuropeptides of the NANC system (CGRP, NKA, SP, and others) [5, 20, 21, 24]. There has been evidence that these reactions in particular can be a link connecting mechanical stimulation with the changes in ion transport responsible for the reaction of hyperpolarization.

Capsaicin, which is a representative of drugs irritating sensory endings, most often causes an increase in the reaction to mechanical stimulation,

but it does not apply to the group incubated with capsaicin (Tab. 2). The increase in the reaction value after the administration of capsaicin may suggest that capsaicin changes secretion of neuropeptides, which leads to dPD changes. Application of capsaicin causes instant increase in the value of electrical potential, which was an affect of C-fibre stimulation and secretion of neuropeptides. Subsequently, the potential stabilized, not returning to the initial value (Fig. 2a). It may be associated with maintaining secretion of neuropeptides at the constant level. The reaction of the caecum wall to Ringer solution (Fig. 2c) and also to capsaicin solvent (Fig. 2b) may be an evidence, confirming this hypothesis. Administration of both these compounds, at the beginning caused an increase in electrical potential followed by its decline down to the control level. Reaction increase during capsaicin administration took place also after blocking of sodium ion transport by amiloride and chloride ions by bumetanide. On the other hand, during incubation in the presence of capsaicin, the reaction value at the time of mechanical stimulation became independent of capsaicin content in the stimulating fluid.

Capsaicin used for incubation of the rabbit caecum lowered the electrical potential value and also the value of reaction to mechanical stimulation by some 40% (Tab. 1). In view of the published data [5], it is likely that it was a result of diminished sensitivity of sensory receptors and a decreased amount of neuropeptides stimulating epithelial cells. Quite possibly, it was due to so extensive secretion of neuropeptides from sensory endings during incubation that it caused a decreased sensitivity of C-fibres to mechanical stimuli. Consequently, neuropeptide secretion decreased after mechanical stimulation. Capsaicin action was irreversible. Initial incubation with capsaicin, followed by incubation in Ringer solution did not change electrophysiological parameters of the caecum (Tab. 1).

Based on the abovementioned data and also on literature data we can assume that the reaction of epithelium of rabbit caecum to capsaicin depends not only on chloride ions but also sodium ions play here a substantial role.

Diversity and variability of the reaction to mechanical stimuli indicate existence of a universal regulatory system, facilitating the intestinal passage.

CONCLUSIONS

1. The isolated rabbit caecum placed in a Ussing apparatus shows a transepithelial electrical potential difference and undergoes a hyperpolarization after stimulation with a mild mechanical stimulus.
2. Studies on transepithelial electrical potential difference with the usage of amiloride, an inhibitor of epithelial sodium channel (and/or bumetanide, an inhibitor of chloride ion transport) revealed that the hyperpolarization reaction in the rabbit caecum, during mechanical stimulation was caused by an increase in transport of sodium (and/or) chlorine).
3. Physiological experiments demonstrate that during mechanical stimulation hyperpolarization reaction of the isolated caecum wall is under influence of C-fibre endings, probably through neuropeptides released by mechanical stimuli from these endings.

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