

## EFFECT OF AMILORIDE AND BUMETANIDE ON IONIC CURRENTS IN THE EPITHELIUM OF CAECUM FROM STARVED RABBITS

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*Effect of amiloride and bumetanide on ionic currents in the epithelium of caecum from starved rabbits.* D. KOSIK-BOGACKA, B. BANACH, T. TYRAKOWSKI. Pol. J. Pharmacol. 2003, 55, 221–226.

The aim of this study was to determine the effect of starvation on the transport of sodium and chloride ions in the epithelium of rabbit caecum. The experiment consisted in measuring transepithelial electrical potential (PD in mV) and the transepithelial electrical potential difference (dPD in mV) of an isolated fragment of rabbit caecum, before and after 4-day-long starvation. The studied tissue was incubated in Ringer solution and subsequently ion transport was modified through incubation in the Ringer solution supplemented with amiloride or/and bumetanide.

It was demonstrated that the values of electrophysiological parameters of the tissue fragments of caecum from starved rabbits were substantially lower than the values for the fragments of control caecum. A similar relationship was observed also in the reaction of this tissue to mechanical stimuli. After the incubation of the caecum tissue fragments in the presence of amiloride or/and bumetanide, the value of transepithelial electrical potential and the sensitivity to mechanical stimuli decreased in both groups studied.

Experimental data presented in this paper indicate that the starvation process has effect on lowering sodium and chloride ion transport and decreasing sensitivity of the epithelium of the caecum to mechanical stimuli.

**Key words:** *amiloride, bumetanide, caecum, rabbit, starvation, Ussing's method, ion transport*

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*Abbreviations: AMI – amiloride, BUMETANIDE – bumetanide, dPD – the difference between maximal stimulated value and control value of PD, MS – mechanical stimulation, NANC – non adrenergic, non cholinergic, PD – transepithelial potential difference (mV)*

## INTRODUCTION

Transepithelial electrical potential (PD) constitutes an important electrophysiological parameter reflecting the functional state of a tissue. It is induced as a result of reabsorption of sodium ions and secretion of chloride ions [5, 15–19, 21–23, 37–39, 42]. Ion transport affects the formation and changes in thickness and viscosity of the mucus layer covering the epithelium of rabbit caecum [22, 23].

It is evident from the literature data that changes in transepithelial ion transport may be caused by neurotransmitters of the non adrenergic, non cholinergic (NANC) system (NKA, SP, CGRP etc.) secreted from the sensory endings in response to mechanical and chemical stimulation [4, 7–10, 27, 31].

The present study may constitute an introduction to discussion on a hypothesis that the amount of neurotransmitters secreted from the sensory endings decreases in subsequent starvation days. An important aim of this study was to examine effect of mechanical and chemical stimuli on PD of the rabbit caecum wall before and after 4-day-long starvation.

## MATERIAL and METHODS

The experiments were carried out on 109 fragments of an isolated caecum wall of a rabbit. The rabbits (36) used for the experiment were divided into two groups:

- Group I (24), with unlimited access to feed around the clock,
- Group II (11), with no feed for three days, receiving only water.

The study consisted in measuring the PD and transepithelial electrical potential difference (dPD) of the fragments of an isolated caecum wall placed in an Ussing apparatus [20]. The readings were taken by EVC 4000 device (manufactured by WPI, USA) and BD recorder 111 (Kipp & Zonnen, the Netherlands) connected to the Ussing apparatus by Ag/AgCl electrodes and agar bridges filled with

KCl solution. Electrical stability of the measuring system was tested by application of solutions on a synthetic cellophane membrane placed in the Ussing apparatus (blank sample).

The rabbits were killed by suffocation with carbon dioxide and the tissues were immediately sampled. The caecum was dissected and its content was removed by gentle rinsing. Subsequently, it was placed in Ringer solution at 36°C, stripped of connective tissue, cut open longitudinally, and divided into pieces. Tissue samples prepared in such a way were incubated and were placed one by one in the Ussing apparatus. The mechanical stimulus (MS) was a jet of the fluid from the chamber of the Ussing apparatus flushing the mucous surface of the caecum. The jet was ejected within 15 s from a nozzle, 1.5 mm in diameter, situated 12 mm from the surface of the studied tissue.

The media used for incubation, filling up chambers of Ussing apparatus, and the stimulation during the experiment were:

- Ringer solution composed of (in mmol/l): Na<sup>+</sup> 147.2, K<sup>+</sup> 4.0, Ca<sup>2+</sup> 4.4, Cl<sup>-</sup> 155.6, HEPES 10.0,
- Ringer solution supplemented with amiloride (AMI) (0.1 mmol/l),
- Ringer solution supplemented with bumetanide (BUMETANIDE) (0.1 mmol/l),
- Ringer solution supplemented with AMI and BUMETANIDE (all supplied by Sigma Chemical Co.).

Statistical hypotheses were verified by chi square test for assessment of the qualitative data and Student's *t*-test for quantitative data ( $p < 0.05$ ). The data were evaluated statistically using "Statgraphics" software.

## RESULTS

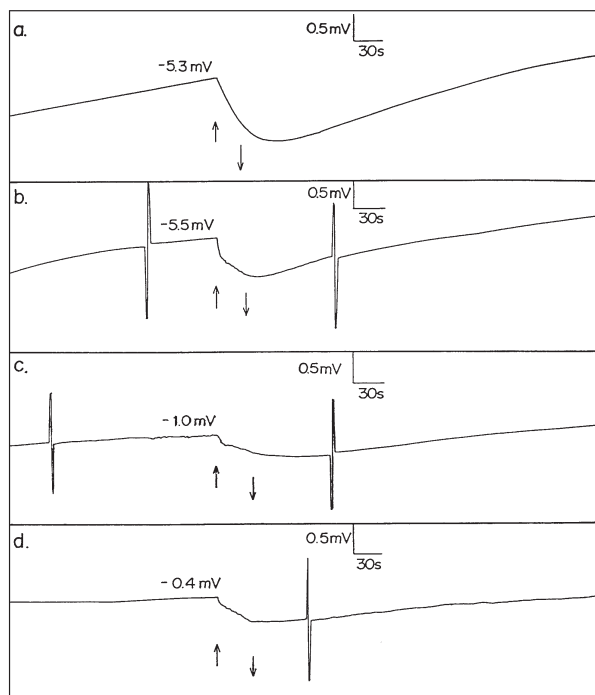
Table 1 shows the basic electrophysiological parameters of the fragments of isolated rabbit caecum before and during starvation. PD and the reaction to MS of the control caecum were by some 80 and 85% higher, respectively, than the values determined in the caecum from starved rabbits.

MS of the control caecum caused hyperpolarization of short duration (Fig. 1a). PD decreased throughout the period of the stimulus action and it regained the initial value within 5 min. MS of the caecum from starved rabbits, incubated in Ringer solution, also caused a short hyperpolarization. After the stimulation, the PD value did not return to the initial value (Fig. 2a). Administration of AMI

**Table 1.** Effect of mechanical and chemical stimuli on electrophysiological parameters of the caecum from control and starved rabbits after incubation in Ringer solution

Caecum (n)	PD (mV)	SM dPD (mV)	AMI dPD (mV)	BUME dPD (mV)	AMI + BUME dPD (mV)
Control n = 24	$-4.8 \pm 0.6$	$-2.0 \pm 0.4$	$-1.4 \pm 0.5$	$-1.1 \pm 0.4$	$-1.5 \pm 0.3$
Experimental n = 11	$-1.0 \pm 0.2^*$	$-0.3 \pm 0.1^*$	$-0.3 \pm 0.1^*$	$-0.3 \pm 0.0^*$	$-0.3 \pm 0.0^*$

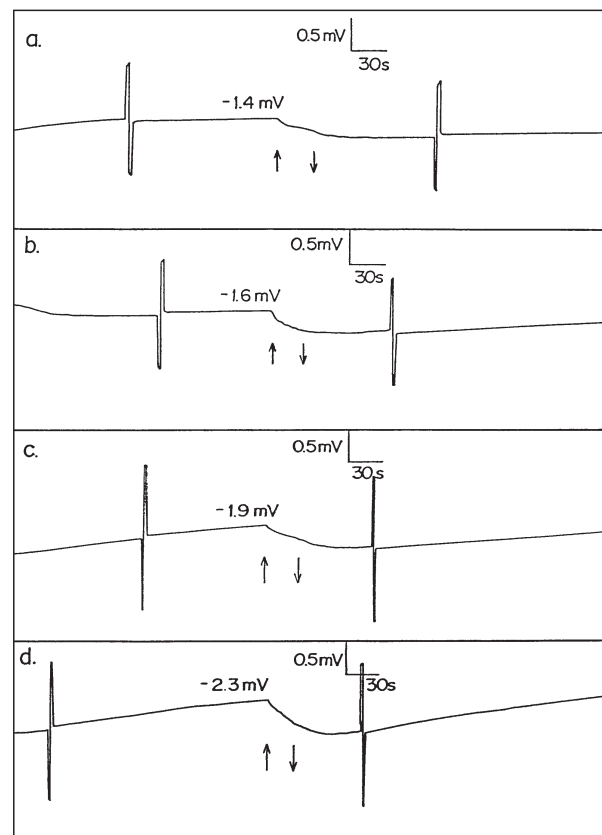
The values represent the mean  $\pm$  SE. Abbreviations: n – number of studied fragments of rabbit caecum, SM – mechanical stimulation, AMI – Ringer solution with amiloride, BUME – Ringer solution with bumetanide dissolved in DMSO, AMI + BUME – Ringer solution with addition of amiloride and bumetanide, \* significantly different in relation to the values in the control group ( $p < 0.05$ )



**Fig. 1.** Changes of transepithelial electrical potential of an isolated rabbit caecum under influence of mechanical stimuli and variable incubation conditions. The intestine was incubated in Ringer solution (a) and in Ringer solution supplemented with: amiloride (b), bumetanide (c), amiloride and bumetanide (d). The mechanical stimulation consisted in rinsing the mucous surface for 30 s with the incubation liquid

or BUME, or both these compounds jointly, did not affect the reaction value in both experimental groups.

Incubation in the presence of AMI (Tab. 2) caused a decrease in PD and the reactivity in both experimental groups compared to incubation of both those tissues in Ringer solution. MS of the control caecum caused reversible hyperpolarization



**Fig. 2.** Effect of the starvation on electrophysiological processes of rabbit caecum. The intestine was incubated in Ringer solution (a) and in Ringer solution supplemented with: amiloride (b), bumetanide (c), amiloride and bumetanide (d). The mechanical stimulation consisted in rinsing the mucous surface for 30 s with the incubation liquid

(Fig. 1b). In some experiments, MS applied to a fragment of caecum from starved rabbits did not cause changes in PD. After the stimulation, the potential did not regain its initial value and remained at a stable level (Fig. 2b).

**Table 2.** Comparison of electrophysiological parameters characterizing the caecum from control and starved rabbits after incubation in Ringer solution supplemented with amiloride

Caecum	PD (mV)	SM dPD (mV)	AMI + BUME dPD (mV)
Control n = 14	-2.5 ± 0.4	-0.6 ± 0.2	-1.0 ± 0.2
Experimental n = 11	-0.5 ± 0.2*	-0.1 ± 0.1*	-0.1 ± 0.0*

For the explanations see Table 1

**Table 3.** Comparison of electrophysiological parameters characterizing the caecum from control and starved rabbits after incubation in Ringer solution supplemented with bumetanide

Caecum	PD (mV)	SM dPD (mV)	AMI + BUME dPD (mV)
Control n = 16	-0.9 ± 0.2	-0.2 ± 0.1	-0.3 ± 0.1
Experimental n = 9	-0.4 ± 0.1*	-0.1 ± 0.1	-0.1 ± 0.0

For the explanations see Table 1

**Table 4.** Comparison of electrophysiological parameters characterizing the caecum from control and starved rabbits after incubation in Ringer solution supplemented with amiloride and bumetanide

Caecum	PD (mV)	SM dPD (mV)
Control n = 14	-0.7 ± 0.2	-0.2 ± 0.1
Experimental n = 9	-0.4 ± 0.2	-0.2 ± 0.0

For the explanations see Table 1

Incubation in the presence of AMI (Tab. 3) caused a decrease in PD, by some 81% and also a decrease in reactivity to MS by some 90% in comparison with incubation in Ringer solution. MS caused hyperpolarization in this group, and after the end of stimulation PD returned to the control value (Fig. 1c). Under these experimental conditions the caecum of starved rabbits demonstrated a decrease in PD by some 60%, while the reaction to MS decreased by some 70%, compared to the incubation in Ringer solution. After the stimulation, the potential did not assume its initial value (Fig. 2c).

Values of electrophysiological parameters of the preparations incubated in the presence of AMI and BUME did not differ from the values obtained for the tissues incubated with BUME in respects of

PD or reactivity (Tab. 4). In both experimental groups, hyperpolarization occurred during the stimulation (Figs. 1d and 2d). After the end of stimulation, the potential returned to its initial value.

## DISCUSSION

Ion transport in various biological systems is possible because of existence of ion pumps in cell membranes. The ionic current induced by ion transport is recorded as changes in PD. A classical system used for measuring dPD *in vitro* has been the Ussing apparatus. It was used for the first time for the studies of electrophysiological parameters of an isolated frog skin [21]. At present this apparatus is commonly used for electrophysiological studies of all epithelial tissues [22, 23, 37–39].

The model of an isolated intestinal tissue of mammals is currently widely used for studies of ion transport in the alimentary tract. In such studies, effect of various pharmaceuticals is tested on an animal model [1, 9, 10, 12–14, 18, 20, 22–24, 26, 29, 34–36, 40].

In the present study, we used the widest part of the large intestine of rabbit, i.e. the caecum. It is the part of the intestine where nutritional substances are absorbed and cellulose and hemicellulose are digested.

PD was the major parameter measured during the present study. This potential reflects the ion transport occurring in the epithelium of the studied tissue and, more precisely, a component of two process: reabsorption of sodium ions and secretion of chloride ions [15, 21–23, 37–39]. The value of this parameter may also reflect influence of physiological stimuli and pharmaceuticals on ion transport occurring in the epithelium of the studied tissue. It is known from our earlier studies that changes in ion transport, induced by MS, such as gentle rinsing with nutritional fluid, are registered as hyperpolarization [22, 23, 37–39].

In the present study, we used starvation as the physiological stimulus. According to the published evidence, the starvation period is characterized, among other factors, by: decreased excretory function of the alimentary tract, reduction of intestinal kinetics, and impairment of digestive and absorptive functions of the alimentary tract [11, 20, 32]. Under the conditions of hunger, certain disorders of the alimentary tract may occur such as diarrhoea or spastic states of intestines.

The presently described experimental data indicate that within 4-day starvation, PD of the rabbit caecum declined by some 79.2%, and the reactivity to MS dropped by about 85% (Tab. 1, Fig. 2a). The decrease in PD of the caecum from starved rabbits was probably caused by blockage of ion channels. On the other hand, the decrease in sensitivity to MS was associated with the decreased secretion of neurotransmitters of the NANC system from sensory endings of C-fibres [25]. Starvation could lead to such strong secretion of neurotransmitters from sensory endings, which could cause a decrease in the sensitivity of C fibres of the caecum to MS. A confirmation of this hypothesis is the fact that after MS, PD of the caecum from starved rabbits did not regain its initial value.

The application of AMI, an inhibitor of sodium ions transport [2, 3, 6, 30, 33, 38], caused a decrease in PD and also in the sensitivity of the tissues to stimuli in both control and experimental group (Tab. 2, Figs. 1b and 2b). The blockage of sodium ion transport in control caecum caused a decrease in PD by some 48% and weakened the reaction to MS by about 70% when compared to incubation in Ringer solution. The same experimental conditions applied to the caecum from starved rabbits caused lowering of PD by some 50% and a drop in the reaction to MS by about 67%. The value of PD of the caecum from starved rabbits was by some 80% lower in comparison with the control. Its sensitivity to the used MS was lower by about 84%.

The incubation of control caecum with an inhibitor of transepithelial chloride ion transport BUMA [28] caused a decrease in the value of transepithelial electrical potential by some 81% and a reduction in the reaction to MS by some 90%, compared to incubation in Ringer solution (Tab. 3, Fig. 1c). Under the same experimental conditions, the caecum from starved rabbits showed PD value lower by some 60%, and the reaction to MS was decreased by 33% (Tab. 3, Fig. 2c). Comparison of both parameters in control and experimental caecum showed that after starvation and after incubation with AMI, the PD value decreased by some 56% and dPD by some 50%.

The application of both transport inhibitors, AMI and BUMA, caused a decrease in PD and the reaction to the stimulation in the control group by some 85% and 95%, respectively (Tab. 4; Fig. 1d). After application of AMI and BUMA for the incubation of the caecum from starved rabbits, the value of PD

declined by some 60% and its reactivity by some 33% compared to control incubation (Tab. 4, Fig. 2d). Comparison of electrophysiological parameters of control and experimental caecum indicated that PD of caecum from starved rabbits was lower by some 43%, while the dPD value was not different from the control value.

## CONCLUSIONS

1. Starvation process causes a decrease in sensitivity of the rabbits caecum to MS.
2. Inhibition of sodium ion transport decreases PD, as well as sensitivity to MS, regardless of whether the animal was starved or not.
3. Chloride ion transport is responsible for PD and reactivity to MS of the wall of the large intestine.
4. Reversibility of hyperpolarization of PD after the action of MS depends on stimulation of afferent sensory endings and the release of neuropeptides, which induce changes of the transepithelial ion transport. Efficiency of this reaction decreases after starvation.

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Received: December 6, 2002; in revised form: March 3, 2003.