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Effect of Acetylcholine on Follicle-Associated Peyer's Patches Epithelium

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ABSTRACT

The study aims to investigate the regulation of tissue barrier functions by endogenous compounds, particularly acetylcholine, in the intestinal epithelium. The epithelium contains gut-associated lymphoid tissue (GALT), Peyer's patches (PP), and specialized follicle-associated epithelium (FAE), which play a crucial role in intestinal function and immune responses. The FAE contains microfollicular cells responsible for phagocytosis, transcytosis, and antigen presentation, allowing for controlled uptake of large particles and initiation of an immunological response. The digestive tract is extensively innervated by cholinergic fibers, and acetylcholine (ACh) is a neuroimmunomodulator of the intestinal mucosa. The cholinergic anti-inflammatory pathway is a local anti-inflammatory response in the intestinal mucosa. Peyer's patches are immune cells that maintain immune tolerance to commensal flora and dietary antigens. The study of barrier functions of the follicle-associated epithelium of Peyer's patches in the rat small intestine under the action of acetylcholine is currently an understudied problem in gut-associated lymphoid tissue physiology. The aim is to investigate changes in the barrier properties of follicle-associated Peyer's patches epithelium in the rat small intestine under the action of acetylcholine.

Keywords: Peyer's Patches, Follicle-Associated Epithelium, Ter, Acetylcholine, Ussing Chamber.

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INTRODUCTION

The study of mechanisms of regulation of tissue barrier functions by endogenous compounds is an urgent problem of modern physiology of visceral systems. The central role in ensuring the functions of tissue barriers is played by epithelial cells, which are united by tight contacts (Günzel & Fromm, 2012). The intestinal epithelium is one of the largest tissue barriers in contact with the environment. It contains gut-associated lymphoid tissue (GALT) represented by mesenteric lymph nodes, isolated lymphoid follicles, and Peyer's patches (PP). PP are covered by specialized follicle-associated epithelium (FAE), whose barrier properties differ from the adjacent villous epithelium (Radloff et al., 2017; Tamagawa et al., 2003). The Peyer's patches epithelial barrier in the rat small intestine undergoes dynamic changes under the influence of various factors (Radloff et al., 2019). These changes play a critical role in intestinal function and immune responses. Peyer's patches are specialized structures of the intestinal mucosa that serve to recognize and present antigens to cells of the immune

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system(Mörbe et al., 2021). The FAE contains microfollicular cells (M cells), which are responsible for the processes of phagocytosis and transcytosis as well as antigen presentation (Owen & Jones, 1974; Takeuchi & Gonda, 2004). Through these processes, the FAE performs controlled uptake of large particles from the intestinal lumen and initiation of an immunological response (Sakhon et al., 2015). The GALT is the most extensive and complex part of the immune system, involving various regulatory mechanisms to effectively fulfil its barrier function. The digestive tract has extensive innervation by cholinergic fibers (Harrington et al., 2010). Acetylcholine (ACh), secreted by the vagus nerve in response to physiological and pharmacological stimulation, is a neuroimmunomodulator of the intestinal mucosa (Wang et al., 2003). In the mucosa of various intestinal segments, activation of nicotinic cholinoreceptors by macrophages, mast cells, and dendritic cells develops a local anti-inflammatory response, which has been defined as the cholinergic anti-inflammatory pathway (Ulloa, 2005). Peyer's patches are a well-organized population of immune cells that play a critical role in maintaining immune tolerance to commensal flora and dietary antigens (Jiao et al., 2020). It has been shown that carbachol administration can influence the state of Peyer's patches FAE during the development of stress (Keita et al., 2010). Nevertheless, the involvement of immune cells in altering paracellular and transcellular permeability under the action of acetylcholine, as a mediator of neurons of various parts of the nervous system, remains an almost unexplored area of gut-associated lymphoid tissue physiology. The study of barrier functions of follicle-associated epithelium of Peyer's patches in rat small intestine under the action of acetylcholine and pharmacological analysis of these effects is currently an understudied problem.

MATERIALS AND METHODS OF RESEARCH

Male Wistar rats (body weight 180-250 g) were used in all series of experiments. The animals were kept in standard laboratory conditions with access to food and water. All procedures were performed by the "Guide for the Care and Use of Laboratory Animals". During preparation, the small intestine with Peyer's patches was excised from the animals, was washed with oxygenated chilled Krebs-Ringer's solution (in mmol/L: 119 NaCl, 5 KCl, 1.2 $MgCl_2 \cdot 6H_2O$, 25 $NaHCO_3$, 0.4 $NaH_2PO_4 \cdot H_2O$, 1.6 $Na_2HPO_4 \cdot 7H_2O$, 1.2 $CaCl_2$) and cut along the mesenteric line. The main criterion for selecting a tissue segment with Peyer's patches for study was their diameter of at least 4 mm. In all segments under study, Peyer's patches were well distinguishable visually and had delineated borders, so no microscopic equipment was required for their detection and excision, which allowed mounting only the section of the intestinal wall with Peyer's patches in the Ussing chamber. The method of registration of electrophysiological parameters in an Ussing chamber was used to study the changes in the epithelial barrier in the follicle-associated epithelium of Peyer's patches in the rat small intestine. The Ussing chamber is a Plexiglas cylinder consisting of two halves with tissue placed between them **(Fig.1)**. To register electrophysiological characteristics, two pairs of electrodes are connected to the chamber: two electrodes for current registration and two electrodes for voltage registration. Each half of the chamber communicates with a vessel containing a solution that washes the epithelial tissue. The reservoir with the solution washing the tissue has an external circuit through which water circulates, coming from the thermostat to maintain and regulate the temperature of the solution used (38°C). Before each experiment, the chamber with a communicating vessel was filled with 5 ml of Krebs-Ringer's solution, which was washed over the tissue under study during the experiment. In the absence of the drug, balancing and voltage compensation between the electrodes were performed. The solution was then drained, sections of Peyer's patches were mounted in an Ussing chamber, and then 5 ml of solution was added to the chamber from the serous and mucosal sides of the epithelium. The glass reservoir had a port for aeration with carbogen (95% O_2 and 5% CO_2). Acetylcholine (Alfa Aesar, USA) was added to the Ussing chamber from the basolateral side, bringing the final concentration in the chamber to 1, 10, or 100 mmol to investigate the dose-dependent effect on Peyer's patches.The first 10 minutes after the preparation installation were reserved for stabilization of electrophysiological parameters and tissue adaptation to the experimental conditions. The potential difference (mV) was registered using voltage electrodes located on the mucosal and serosal sides of the tissue, respectively. Short-circuit current (μA/cm2) was studied in the mode of short-term fixation of voltage at 0 mV and registration of the current value by electrodes. To determine the value of transepithelial resistance in the mode of current fixation at a value of 10 μA, the voltage deflection was recorded. Transepithelial resistance was calculated according to Ohm's law: $R = \Delta U / \Delta I$.

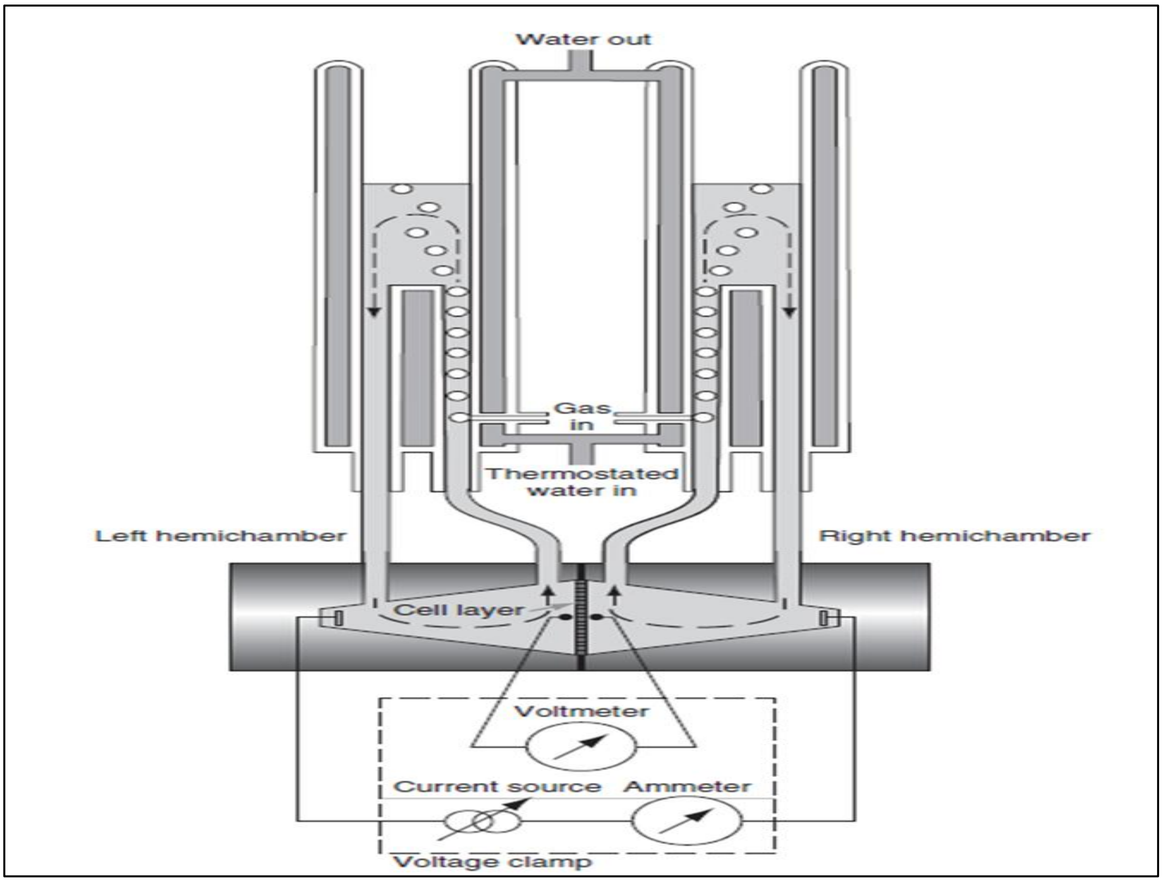

Figure 1. Schematic diagram of Ussing's chamber.

STATISTICAL PROCESSING OF THE OBTAINED RESULTS

Statistical processing of the data was performed in the GraphPad Prism v8 programme (GraphPad Software, USA). Two-factor analysis of variance was used to process electrophysiological indices of the Peyer's patches with normal distribution. The D-Agostin-Pearson test was used to assess the normality of the data. The level of statistical significance was accepted as $p < 0.05$. Data are presented as mean ± standard error of the mean.

RESULTS AND DISCUSSION

In the control group, transepithelial resistance (TER) and short-circuit current remained stable throughout the experiment (n=20). The initial value of TER for the control group at the beginning of registration was 79 Ohm-cm2, the "short circuit" current - 29 μA, which corresponds to the data obtained earlier (Markov et al., 2016) The addition of acetylcholine at a concentration of 1 mmol to the washing solution did not affect the value of TER (n=10). It was 81 Ohm-cm2 and remained stable throughout the entire recording period (Fig. 2A).

The "short-circuit" current, as a reflection of active ion transport through the follicle-associated epithelium, was sensitive to the action of acetylcholine. After the addition of acetylcholine in the solution at a concentration of 1 mmol a gradual decrease in the value of this parameter was observed, which by the 20th minute decreased by 41 % and significantly differed from the parameters of the control tissue (n=10; $p<0.05$, two-factor dispersion analysis). It is worth paying attention to the dynamics of the change in the "short circuit" current. A decrease in the value of this parameter was observed almost immediately after acetylcholine application, which lasted about 20 minutes. Further, the "short circuit" current had the lowest value, reaching a plateau, which lasted about 20 minutes. Starting from the 40th minute, a gradual recovery of the "short-circuit" current value to the initial values was detected (Fig. 2B).

Increasing the concentration of acetylcholine in the washing solution, tenfold (10 mmol) changed both electrophysiological parameters. After the addition of acetylcholine, a significant increase in TER of 15% (n=11, $p<0.01$, two-factor analysis of variance) was immediately recorded (Figure 3A). This effect was observed for 25 minutes of incubation, and then a return to initial values was noted. The "short-circuit" current showed similar changes to the data using 1 mmol acetylcholine, but the dynamics of these changes are different.

This parameter decreased immediately after acetylcholine applications, reaching statistically significant differences by the 15th minute of recording by 42% ($p<0.05$, two-factor analysis of variance). No period of steady decrease in the "short circuit" current was observed during the action of 10 mmol acetylcholine. At the 20th minute, there was a recovery of this parameter (Fig. 3B).

The use of acetylcholine at a concentration of 100 mmol caused significant changes in the electrophysiological parameters of Peyer's patches. The addition of acetylcholine (100 mmol) resulted in a significant 30% decrease in TER by the 5th minute of recording. (n=13, $p<0.001$, two-factor analysis of variance). This effect continued throughout the incubation period (Fig. 4A). Simultaneously, a significant decrease in the "short circuit" current was found, which was also irreversible until the end of chamber registration ($p<0.01$, two-factor analysis of variance) (Fig. 4B).

Prolonged incubation (60 min) of tissue with a physiologically active substance requires analysis of its direct action, as well as possible changes of this compound and reduction of active concentrations. Acetylcholine has a half-life of about 1-2 min. Its application in increasing concentrations allows prolonging its action and evaluating its effect on the object under study. In the conducted experiments, the sequential application of increasing concentrations of acetylcholine allowed for to comparison of its effect. In addition, it should be taken into account that there is a nonspecific cholinesterase in the tissue (Maharshak et al., 2013), which can also reduce the concentration of acetylcholine. It can be assumed that this effect would not be significant under the given conditions of the experiment. The volumes of solution that wash in from the mucosa and serosa sides are 5 ml. The object itself is limited to the aperture area of the Ussing chamber, which is 0.13 mm2. The ratio of the volume of the washing solution to the possible diffusion of tissue acetylcholinesterase is not comparable in importance.

Nevertheless, in assessing the physiological effects of acetylcholine, emphasis was placed on the recorded changes in the first half of the incubation time. The analysis of changes in TER does not give a clear picture of the nature of acetylcholine action. At a concentration of 1 mmol, there is no change in TER. When increased to 10 mmol, an increase is registered, and when 100 mmol is added, a decrease in this parameter is registered, which suggests the inclusion of different mechanisms of transcellular and paracellular transport when the concentration of acetylcholine is increased.

The "short-circuit" current shows uniqdirectional changes when all concentrations are applied, which confirms its inhibitory effect on active ion transport through the follicle-associated epithelium of Peyer's patches. Only the dynamics of the current changes differ. The application of acetylcholine at a concentration of 1 mmol gives the greatest effect and a prolonged decrease in the "short-circuit" current. At the action of 10 mmol, there is an equal reduction in amplitude of current, but less long in time. The concentration of 100 mmol causes a significant decrease in this parameter throughout the whole incubation period. Analysing the obtained data from the point of view of subsequent pharmacological analysis, the concentration of 10 mmol should be recognized as the most promising for such an experiment. The simultaneous change in TER and short-circuit current, taking into account the processes they reflect, indicates a simultaneous change in paracellular and transcellular transport in the follicle-associated epithelium of Peyer's patches of rat small intestine.

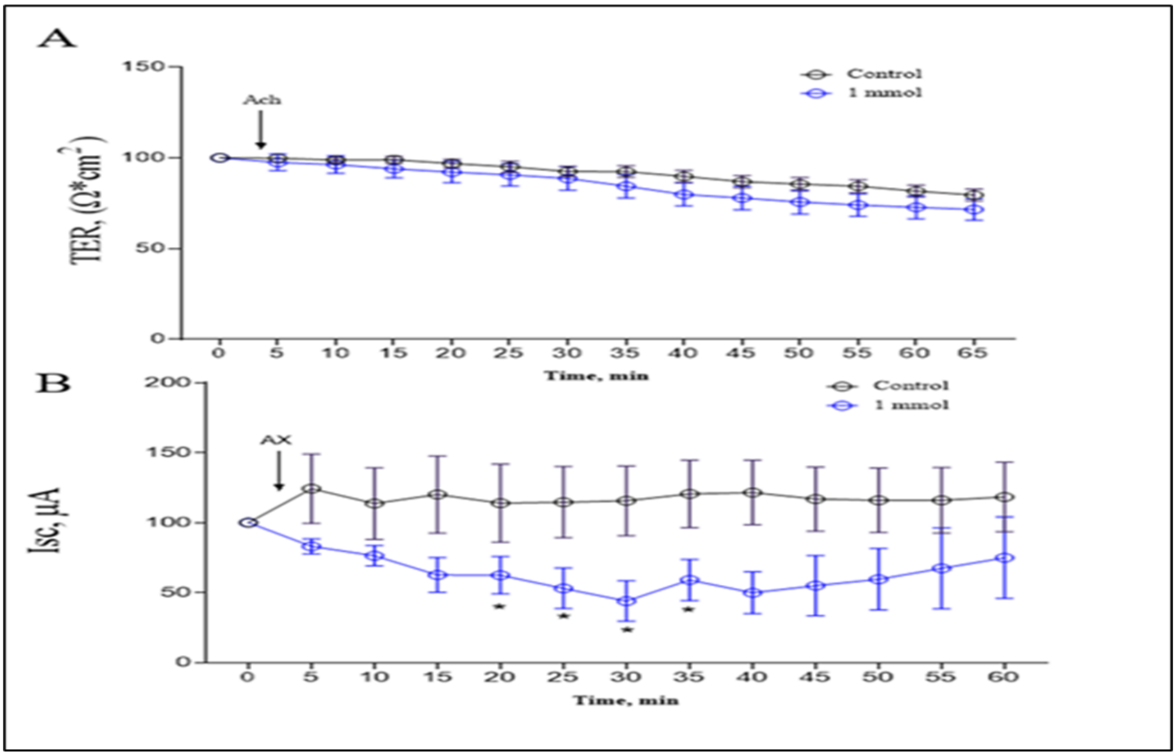

Figure 2 Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 1 Mmol.

A) Transepithelial resistance

B) Short-circuit current (* - $p<0.05$, two-factor analysis of variance)

The arrow indicates the addition of acetylcholine. Baseline values are taken as 100%.

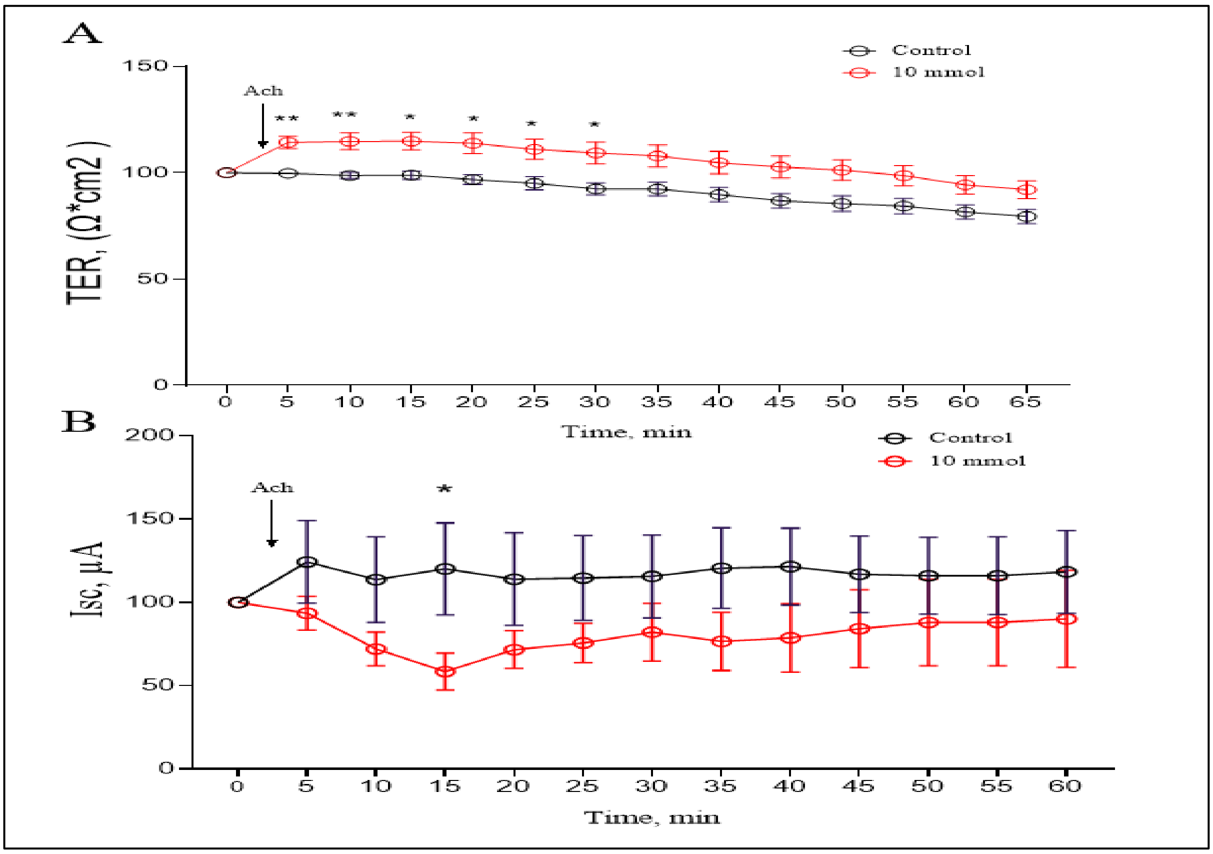

Figure 3 : Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 10 mmol.

A) Transepithelial resistance (* - p<0.05, ** - p<0.01, two-factor analysis of variance)

B) Short-circuit current (* - p<0.05, two-factor analysis of variance)The arrow indicates the addition of acetylcholine.Baseline values are taken as 100%.

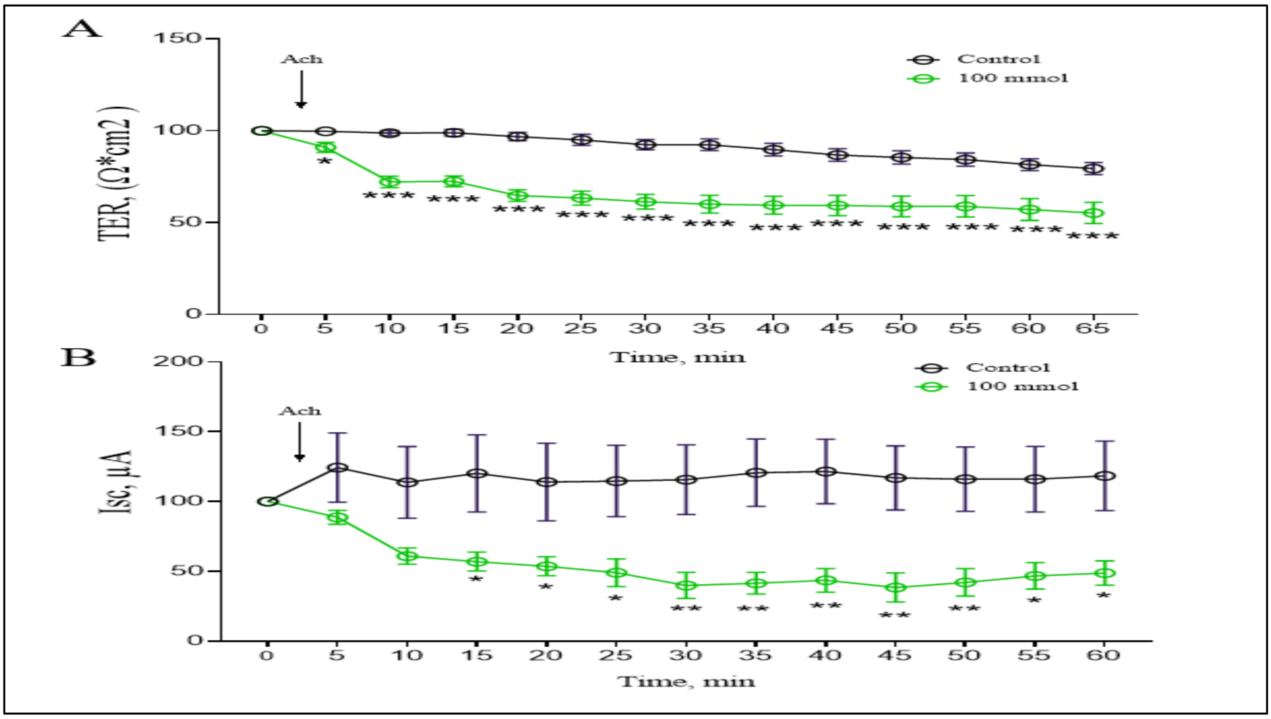

Figure 4 : Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 100 mmol.

A) Transepithelial resistance (* - $p < 0.05$; *** - $p < 0.001$, two-factor analysis of variance)
B) Short-circuit current (* - $p < 0.05$; ** - $p < 0.01$, two-factor analysis of variance)

The arrow indicates the addition of acetylcholine.Baseline values are taken as 100%.

CONCLUSION

The study aimed to analyze the physiological effects of acetylcholine on the follicle-associated epithelium of Peyer's patches of the rat small intestine. The initial value of transepithelial resistance (TER) and short-circuit current remained stable in the control group. The addition of acetylcholine at a concentration of 1 mmol to the washing solution did not affect the value of TER, but the "short-circuit" current was sensitive to the action of acetylcholine. The "short-circuit" current decreased by 41% by the 20th minute, significantly different from the control tissue. Increasing the concentration of acetylcholine in the washing solution tenfold (10 mmol) changed both electrophysiological parameters.

The addition of acetylcholine resulted in a significant increase in TER of 15% and a significant decrease in the "short circuit" current. The study also found that the presence of a nonspecific cholinesterase in the tissue could also reduce the concentration of acetylcholine. The study focused on the recorded changes in the first half of the incubation time, focusing on the effects of acetylcholine on the follicle-associated epithelium. The "short-circuit" current showed unidirectional changes when all concentrations were applied, confirming its inhibitory effect on active ion transport through the follicle-associated epithelium of Peyer's patches. The concentration of 10 mmol should be recognized as the most promising for subsequent pharmacological analysis.

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