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**Changes in the epithelial barrier in the follicle-associated
epithelium of Peyer's patches in the rat small intestine**

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In

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ قَالَ تَزْرَعُونَ سَبْعَ سِنِينَ دَأَبًا فَمَا حَصَدْتُمْ فَذَرُوهُ فِي

سُنْبُلِهِ إِلَّا قَلِيلًا مِّمَّا تَأْكُلُونَ ﴾

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Dedication

In the Name of Allah, the Most Gracious, the Most Merciful

All praise is due to Allah, whose grace, guidance, and mercy enabled me to complete this academic journey.

To my beloved parents, my dear mother and father, who, after Allah Almighty, have been the greatest source of my strength and success. They taught me the value of knowledge, patience, and perseverance, and surrounded me with their love, care, and sincere prayers. May Allah reward them abundantly and grant them long, blessed lives.

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Alhamdulillah Rabbil-'Alamin.

Abdulrahman

Supervision Certificate

We certify that abstract of the thesis entitled (**Changes in the epithelial barrier in the follicle-associated epithelium of Peyer's patches in the rat small intestine**) which was prepared and previously discussed by (**Abdulrahman Salim Qatia Alazzamee**) at the Department of General Physiology, Faculty of Biology, and Federal State Budgetary Educational Institution of Higher Education "Saint-Petersburg State University" ", under our supervision in the Department of Life Sciences, Collage of Science, University of Al-Qadisiyah, Iraq as part of the requirements for the procedures for equivalence of the **Aspirantura (PhD) degree**.

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Summary

This study aimed to investigate changes in the barrier properties of the follicle-associated epithelium of Peyer's patches in the small intestine of rats under the action of acetylcholine and the n-cholinergic receptor blocker mecamlamine.

Peyer's patches are specialized structures of the intestinal mucosa. The epithelial barrier of Peyer's patches of rats undergoes dynamic changes under the influence of various factors. These changes play a crucial role in intestinal function.

The study of the barrier functions of follicle-associated epithelium of Peyer's patches under the influence of acetylcholine ACh and the pharmacological analysis of these effects is currently an understudied problem. This can be determined using pharmacological analysis, represented by the use of ACh blocker (mecamlamine) nAChRs. Thus, since there is non-specific tissue acetylcholine and its activity in our experiment could not be determined, we took different concentrations (1 mmol, 10 mmol - 100 mmol) to eliminate the possible effects of acetylcholine.

Present study was conducted in the Department of General Physiology, College of Science, Saint Petersburg State University, in cooperation with the Resource Centre "Development of Molecular and Cellular Technologies" of SPbU, in the city of Saint Petersburg, during the period from April 2022 until the end of December 2022; the total number of samples was (n=54). The main criterion for selecting a tissue segment with Peyer's patches for study was a diameter of at least 4 mm². administration of ACh and the nAChRs blocker by Ussing chamber.

Peyer's patches after incubation in the chamber were placed in formalin solution for histological examination by using Alcian blue stain, electrophysiological characteristics estimated by Ussing chamber, while Sodium fluorescein (376 Da) was used to study the paracellular permeability of the Peyer's patches epithelium.

The results of electrophysiological parameters of rat Peyer's patches which included transepithelial resistance (TER) and "short-circuit" current in Peyer's patches preparations. The addition of acetylcholine at a concentration of (1 mmol) no effects on TER and significant decreases short-circuit" current, while Increasing the concentration of acetylcholine at (10 mmol) changed both electrophysiological parameters, a significant increase in TER and decreased of short-circuit" current. On another hand, addition of cetylcholine at (100 mmol) decreased both electrophysiological parameters (TER and short-circuit" current).

In order to verify the action of acetylcholine at a concentration of (10 mmol), the experiment studied the secretion of mucin by goblet cells of the mucous membrane. It is known that acetylcholine causes mucin secretion in the small intestine. The study showed an increase in mucin secretion by goblet cells under the action of acetylcholine, which allows the obtained electrophysiological data to be verified. Mecamylamine under the action of acetylcholine, the value of mecamylamine, i.e., the current, does not change. With the sequential application of mecamylamine and acetylcholine, it was observed that the effect of acetylcholine was blocked.

In the control group, the permeability values were normal. The application of acetylcholine causes a significant increase in intercellular permeability. Mecamylamine does not cause a change in intercellular permeability. The use of mecamylamine with acetylcholine causes a blockade of the action of acetylcholine.

Based on our results, we have proven that the non-specific N-cholinergic receptor blocker mecamylamine blocks changes in electrophysiological parameters, as well as changes in the diffusion of sodium fluorescein in the FAEs of Peyer's patches. Considering that n-cholinergic receptors in the intestinal mucosa are located in immunocompetent cells, this indicates the involvement of these cells in the regulation of the barrier properties of Peyer's patches under cholinergic influence.

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List of Abbreviations

Abbreviation	Full Name
ACh	Acetylcholine
α -BGT	α -bungarotoxin
$\alpha 7$ -nAChR	$\alpha 7$ nicotinic acetylcholine receptors
AICD	activation-induced cytidine deaminase
ANS	autonomic nervous system
APCs	antigen presenting cells
BCAF	B cell activating factor
BCR	B-cell receptor
cDC1	conventional dendritic cells 1
cDC2	conventional dendritic cells 2
CSR	class switching recombination
ENS	enteric nervous system
Ep	epithelium
FAE	follicle-associated epithelium
GALT	Gastro-Associated lymphoid Tissue
GC	goblet cells
GP2	glycoprotein 2
HEVs	high endothelial venules
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IECs	intestinal epithelial cells
IL	interleukin
ISC	intestinal stem cells
LPLs	lipoprotein lipase
M2R	muscarinic receptor 2
MALT	mucosa-associated lymphoid tissue

MEC	mecamylamine
MHC	major histocompatibility complex
NF	nuclear factor
NK-1R	neurokinin 1 receptor
NMDA	N-methyl-D-aspartate
nAChRs	nicotinic acetylcholine receptors
PIL	proliferation-inducing ligand
PNS	parasympathetic nervous system
PP	Peyer's patches
PRC2	polycomb repressive complex 2
SBV	space between intestinal villi
SED	subepithelial dome
SEC	subepithelial canopy
SHM	somatic hypermutation
SNS	sympathetic nervous system
TER	Transepithelial resistance
TNF	tumor necrosis factor
ZO	zonula occludens

Saint-Petersburg State University

Alazzamee Abdulrahman Salim Qatiah



Abstract

Graduation thesis

Changes in the epithelial barrier in the follicle-associated epithelium of Peyer's patches in the rat small intestine

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The work was carried out at the Department of General Physiology, Faculty of Biology, and Federal State Budgetary Educational Institution of Higher Education "Saint-Petersburg State University"

**Scientific supervisors: Markov Alexander Georgievich,
Doctor of Biology, Professor**

Structure and volume the graduate thesis.

Is outlined on 82 pages of typewritten text, consists of an introduction, literature review, description of materials and methods of research, results, discussion, conclusions and a list of literature, including 144 sources. The work contains Eight figures.

GENERAL DESCRIPTION OF THE WORK

Chapter One: Introduction and Literature Review

1.1. 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 and 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 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 and Jones, 1974; Takeuchi and 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 and dendritic cells develops a local anti-inflammatory response, which has been defined as the cholinergic anti-inflammatory pathway (Ulloa, 2005).

Mecamylamine is a well-established, non-selective, non-competitive antagonist of nicotinic acetylcholine receptors (nAChRs). It acts primarily as a channel blocker, inhibiting ion flow through activated nicotinic receptors and thereby reducing cellular responses mediated by acetylcholine. Because of its ability to inhibit a broad range of nAChR subtypes, mecamylamine is widely used as a pharmacological tool to investigate the involvement of nicotinic cholinergic signaling in physiological and cellular responses (Nickell *et al.*, 2013).

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.

1.1.1. The Aims of study

Thus, **the aim** of this study was to investigate changes in the barrier properties of follicle-associated Peyer's patches epithelium in the rat small intestine under the action of acetylcholine and the nAChRs blocker mecamylamine.

1.1.2. Objectives of the study:

1. Study of electrophysiological parameters of follicle-associated epithelium of patches of rat small intestine under the action of different concentrations of acetylcholine.

2. Pharmacological analysis of changes in transepithelial resistance and "short-circuit" current in Peyer's patches preparations using the non-selective n-choline blocker mecamylamine.

3. Determination of paracellular permeability of follicle-associated epithelium of Peyer's patches of rat small intestine during sequential administration of the n-choline blocker mecamylamine and acetylcholine using sodium fluorescein

1.2. Literature review

1.2.1. The immune system associated with mucous membranes; Peyer's patches, their cellular composition and functions

Peyer's patches are a group of well-organized lymphoid follicles located in the lamina propria and submucosa of the distal small intestine—the ileum and jejunum, and sometimes the duodenum. Almost 50% of these areas are located in the distal ileum. These patches represent a specialized immune system of the intestine that helps recognize antigens and produce antibodies. They grow and increase in number to a maximum between the ages of 15 and 25, after which their number decreases with age (Cornes, 1965).

The high density of cells in this lymphoid cluster forms protruding nodules that are visible from the peritoneal side of the dissected gastrointestinal tract.

Peyer's patches are organized into lymphoid follicles that are predominantly composed of B cells, with distinct T-cell zones and various antigen-presenting and innate immune cells. B lymphocytes are mainly localized within the follicles and germinal centers, whereas T lymphocytes are predominantly found in the interfollicular and parafollicular regions. The follicle-associated epithelium (FAE) contains specialized microfold (M) cells that sample and transport luminal antigens across the intestinal epithelium to underlying antigen-presenting cells, particularly dendritic cells. This antigen-transport pathway facilitates antigen processing and presentation and contributes to the initiation of adaptive mucosal immune responses within Peyer's patches (Reboldi & Cyster, 2016; Park et al., 2023).

A distinctive feature of Peyer's patches is that these lymphoid follicles are surrounded by epithelium known as follicle-associated epithelium (FAE) (Owen & Jones, 1974). This epithelium contains a reduced number of intestinal villi, a thin

layer of mucous membrane, and is characterized by the presence of epithelial M cells called microfollicular cells. (Onori *et al.*, 2001) These M cells are antigen-presenting cells of Peyer's patches and thus contribute to their absorption and transport through the intestinal lumen into the bloodstream. The porous nature of the basal lamina of FAE aids in this process of antigen absorption (Takeuchi & Gonda, 2004).

1.2.2. Cholinergic regulation of intestinal functions

The innervation of the gastrointestinal tract includes the external autonomic nervous system (ANS) and the internal enteric nervous system (ENS). The corresponding neurotransmitter receptors are widely distributed in various intestinal cells. The intestinal sympathetic nerve innervates various segments of the intestine, regulating the activity of smooth muscle in the gut, and reducing mucosal secretion (Browning and Travagli, 2014). The intestinal vagus regulates the motility of the small and large intestines and controls gastric motility and secretion (Bohórquez *et al.*, 2015; Costa *et al.*, 2000). Regulation of the sympathetic and parasympathetic pathways of the gastrointestinal tract plays a crucial role in the regeneration of the intestinal mucosa and immune response.

The influence of parasympathetic neurotransmitters and receptors on barrier function has been demonstrated. Acetylcholine (ACh) mediates increased colon permeability in stress-induced barrier dysfunction in rats, while muscarinic receptor antagonists block increased colon permeability under stress conditions (Gareau *et al.*, 2007). Activation of M3 receptors increases the transport of macromolecules into mouse and human CEC intestinal epithelial cells (Cameron and Perdue, 2007). However, the protective effect of muscarinic receptors on the maintenance of the intestinal epithelial barrier has been observed under different conditions. activation of M1 or M3 muscarinic receptors counteracts the

destruction of the single-layer epithelial barrier of the intestine induced by inflammatory mediators (Khan *et al.*, 2014; Uwada *et al.*, 2017). In addition, nicotinic receptors influence the maintenance of intestinal epithelial barrier integrity, particularly $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ -nAChR). This receptor has been shown to counteract changes in rat tight junction proteins and increased intestinal permeability caused by LPS (Zhang and Li, 2012; H. Zhou *et al.*, 2013).

The importance of nAChRs in modulating biological functions lies in their ability to translate the binding of an endogenous agonist, such as acetylcholine (ACh), into conformational changes that open the ion channel, allowing ion flow and ultimately triggering a cellular response (Albuquerque *et al.*, 2009)

All ligand-activated ion channels have similar architecture and functions. First, their constituent proteins are necessarily transmembrane to create a hydrated receptor channel that is also permeable to individual ions. Second, ion channel receptors are in a constant equilibrium between open and closed states. Therefore, these receptors must contain primary structural components that respond to and are regulated by the presence of external compounds, such as activators (agonists), inhibitors (antagonists), or compounds that alter the effectiveness of these agents. Understanding of the molecular mechanisms underlying these fundamental aspects of nAChR biology has advanced rapidly in recent years, reflecting the dynamic growth of our knowledge of how they function and how they participate in normal and pathological physiology (Changeux and Edelstein, 2005).

Mecamylamine is a secondary amine belonging to an old class of drugs

known as “ganglionic blockers.” MEC non-selectively and non-competitively inhibits both peripheral and central nAChRs (Giniatullin *et al.*, 2000), acting as an open channel blocker (VanMeter, 1984; Varanda *et al.*, 1985). MEC reduces the duration of the open state of the channel by interacting with the open channel site of nAChR (Young *et al.*, 2001). Sympathetic ganglionic blockade, caused by stabilization of postsynaptic membranes against AC excitation, leads to vasodilation, increased peripheral blood flow, and decreased cardiac output, resulting in a reduction in blood pressure (Young *et al.*, 2001.; Young *et al.*, 2001).

Green *et al.* (2003) showed that enteric nerves modulate the uptake of enteropathogens in Peyer’s patches of the porcine jejunum, and several studies in rats have shown that changes in the mucosal barrier in the epithelium of villi (BE) are mainly associated with corticotropin-releasing hormone (CRH), mast cells, and cholinergic neurons. (Castagliuolo *et al.*, 1998; Saunders *et al.*, 1997; Söderholm *et al.*, 2002) Peripheral administration of CRH mimics stress-induced changes in permeability in rats (Pothoulakis *et al.*, 1998; Santos *et al.*, 1999), and intraperitoneal administration of a CRH antagonist suppresses stress-induced disturbances. (Saunders *et al.*, 2002) Furthermore, increased permeability after stress is absent in rats with mast cell deficiency (Söderholm *et al.*, 2002), indicating the importance of mast cells for the barrier function of the mucosa. Mast cells are often located near neurons and are activated by neuropeptides such as CRF, substance P, and acetylcholine (Stead *et al.*, 1989).

Little is known about the action of AC in Peyer's patches. Immunohistochemistry of pig follicles showed cholinergic innervation and expression of muscarinic receptor 2 (M2R) (Kulkarni-Narla *et al.*, 1999; Ma *et al.*, 2007).

1.2.3. The role of acetylcholine in regulating the functions of the intestinal epithelial barrier

APCs perform a more complex task in the epithelial barrier of the intestine than in the epithelial barrier of the skin and mucous membranes. They support paracellular transport of water, ions (e.g., Na⁺, Ca²⁺, Mg²⁺, and Cl⁻), and nutrients (e.g., glucose and protein), and prevent the translocation of microorganisms (Turner *et al.*, 2014). PCs also prevent apoptosis of intestinal epithelial cells and protect the intestinal epithelial barrier (Kuo *et al.*, 2019). Na⁺ transport is necessary for nutrient absorption. For example, Na⁺-glucose cotransport through tight junctions is vital for maintaining Na⁺ concentrations inside and outside the intestinal epithelial barrier, which ensures nutrient recycling. In the case of tight junction deficiency, the intracellular and extracellular Na⁺ concentration gradient in the intestinal epithelial barrier is insufficient to support transcellular Na⁺-glucose cotransport, which affects nutrient absorption (Turner *et al.*, 2014). Thus, a deficiency of tight junctions can lead to impaired intestinal epithelial absorption and abnormal secretory function.

Recently, many studies have focused on the role of the vagus nerve in inflammatory responses. Stimulation of the vagus nerve suppresses the release of proinflammatory cytokines in endotoxemic mice (Borovikova *et al.*, 2000). This new function of the vagus nerve has been termed the cholinergic anti-inflammatory pathway (Ulloa, 2005). Stimulation of the vagus nerve can release acetylcholine, which interacts with the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on macrophages, suppressing inflammation, confirming the necessity of $\alpha 7$ nAChR for this pathway (Wang *et al.*, 2003). The precise cellular mechanism may involve Jak-2/Stat3 signaling and inhibition of NF- κ B activity (de Jonge *et al.*, 2005; Tracey, 2007).

In addition to suppressing systemic inflammation, the vagus nerve is also a major regulator of inflammation confined to the intestine. The vagus nerve communicates with the enteric nerve, which innervates the gastrointestinal tract (Blackshaw *et al.*, 2007). Stimulation of the vagus nerve has been shown to reduce intestinal inflammation in trinitrobenzenesulfonic acid-induced colitis (Meregnani *et al.*, 2011). Further studies have shown that the $\alpha 7$ nAChR agonist can also activate the cholinergic anti-inflammatory pathway, achieving a protective effect in a model of postoperative ileus in mice (The *et al.*, 2007). Recent studies have shown that vagus nerve stimulation can prevent systemic organ dysfunction in trauma and hemorrhagic shock (Levy *et al.*, 2013).

The results also showed that vagus nerve stimulation suppressed NF-kB activity in the intestinal mucosa, thereby inhibiting the increased level of MLCK in endotoxemia, preventing tight junction opening and intestinal barrier dysfunction. α -BGAT reversed the protective effect of vagus nerve stimulation. All these data support the idea that vagus nerve stimulation protects the barrier function of tight junctions in endotoxemic mice, and $\alpha 7$ nAChR plays an important role in this process (H. Zhou *et al.*, 2013).

Recent studies have shown that vagus nerve stimulation protects the intestinal barrier not by suppressing systemic inflammation, but by modulating local inflammation in the intestine (Costantini *et al.*, 2010). The microenvironment in the mucosa and submucosa was innervated by the cholinergic enteric nerve and contained a large number of immune cells (Matteoli and Boeckxstaens, 2013). Further studies also showed that the protective effect of vagus nerve stimulation may not be limited to macrophages, but may also affect other types of cells expressing $\alpha 7$ nAChR, such as mast cells, endothelial cells, and dendritic cells (Van Der Zanden *et al.*, 2009). Thus, the protective effect of vagus nerve stimulation on

tight junction integrity in endotoxemic mice may be due not only to macrophages but also to other cells expressing $\alpha 7$ nAChR.

MAIN CONTENTS OF WORK

Chapter Two : Material and Methods

2. Materials and Methods of Research

2.1. Laboratory animal and samples collection

A total of 54 tissue samples ($n = 54$) were obtained from male Wistar rats weighing 180–250 g and approximately 6–8 weeks old. The animals were maintained under standard laboratory conditions with free access to food and water. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

The experimental design consisted of several experimental series in which Peyer's patch-containing segments of the small intestine were prepared for electrophysiological, permeability, and histological analyses. The overall sequence of animal preparation, tissue collection, experimental treatment, and subsequent analyses is summarized in Figure 2-1.

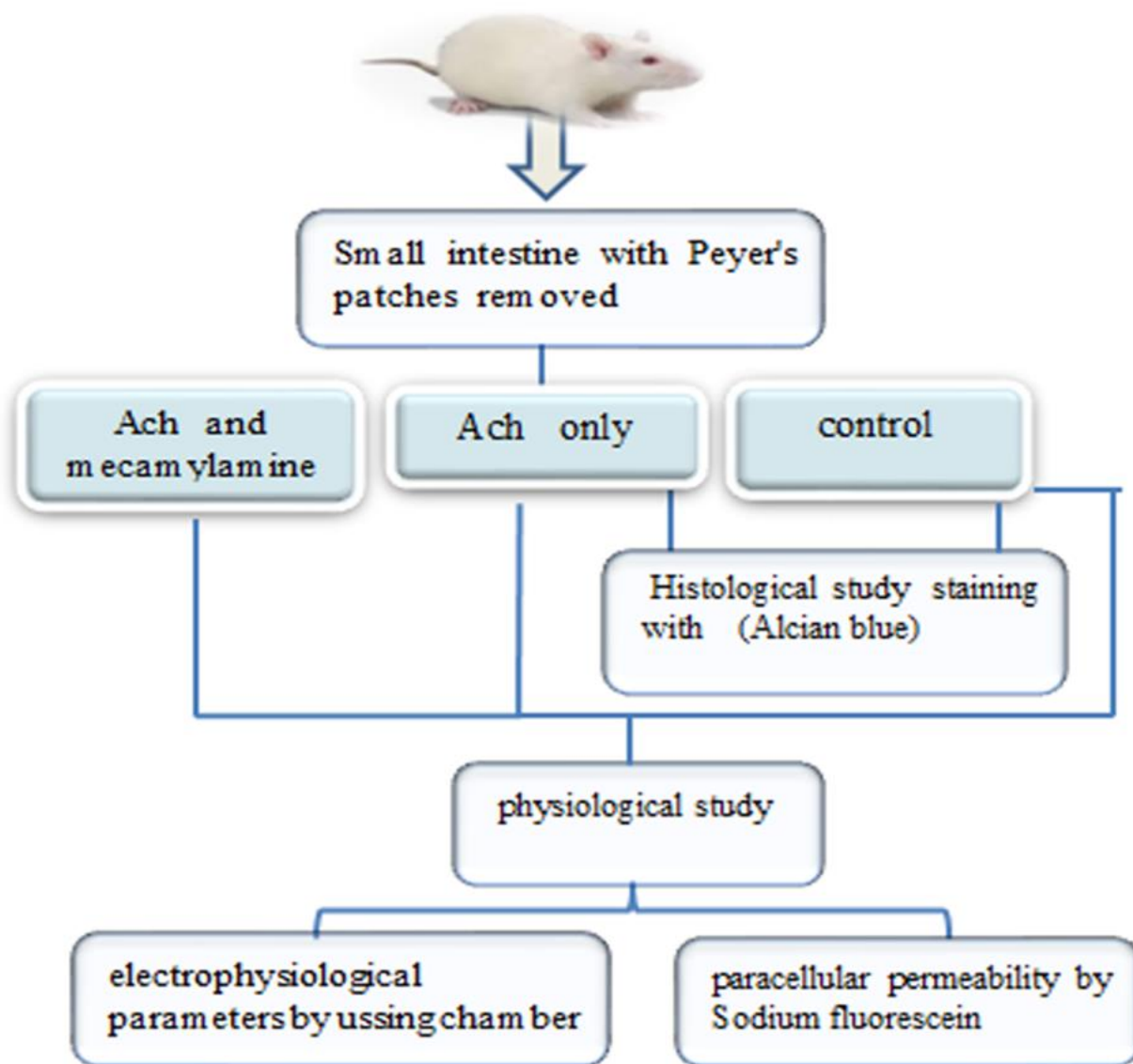


Figure (2-1). Experimental design

During sample preparation, the small intestine containing Peyer's patches was excised from the animals and washed with oxygenated, chilled Krebs-Ringer's solution containing (mmol/L): 119 NaCl, 5 KCl, 1.2 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 25 NaHCO_3 , 0.4 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1.6 $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, and 1.2 CaCl_2 . The intestine was then opened along the mesenteric line.

The main criterion for selecting tissue segments containing Peyer's patches was a patch diameter of at least 4 mm. The Peyer's patches were visually distinguishable and had clearly defined borders; therefore, microscopic equipment was not required for their identification and excision. Only the intestinal wall section containing the Peyer's patch was mounted in the Ussing chamber for subsequent experiments.

All stages of the study were performed using equipment available at the Resource Centre "Development of Molecular and Cellular Technologies" of Saint Petersburg State University.

2.2. Registration of electrophysiological characteristics in an 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 (Figure 2-1). 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 washing 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 communicating vessel was filled with 5 ml of Krebs-Ringer's solution, which was washed on the tissue under study during the experiment. In the absence of the drug, balancing and voltage compensation between the electrodes

was 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% O₂ and 5% CO₂).

The experiment was divided into two stages. In the first stage, 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 in order to investigate the dose-dependent effect on Peyer's patches. In the second step, mecamylamine, a non-selective nAChRs antagonist at a final concentration of 1 mmol, was added to the basolateral side 30 min before acetylcholine administration.

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 ($\mu\text{A}/\text{cm}^2$) 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 (Clarke, 2009): $\mathbf{R = \Delta U / \Delta I}$

Some of the Peyer's patches after incubation in the chamber were placed in formalin solution for light microscopy.

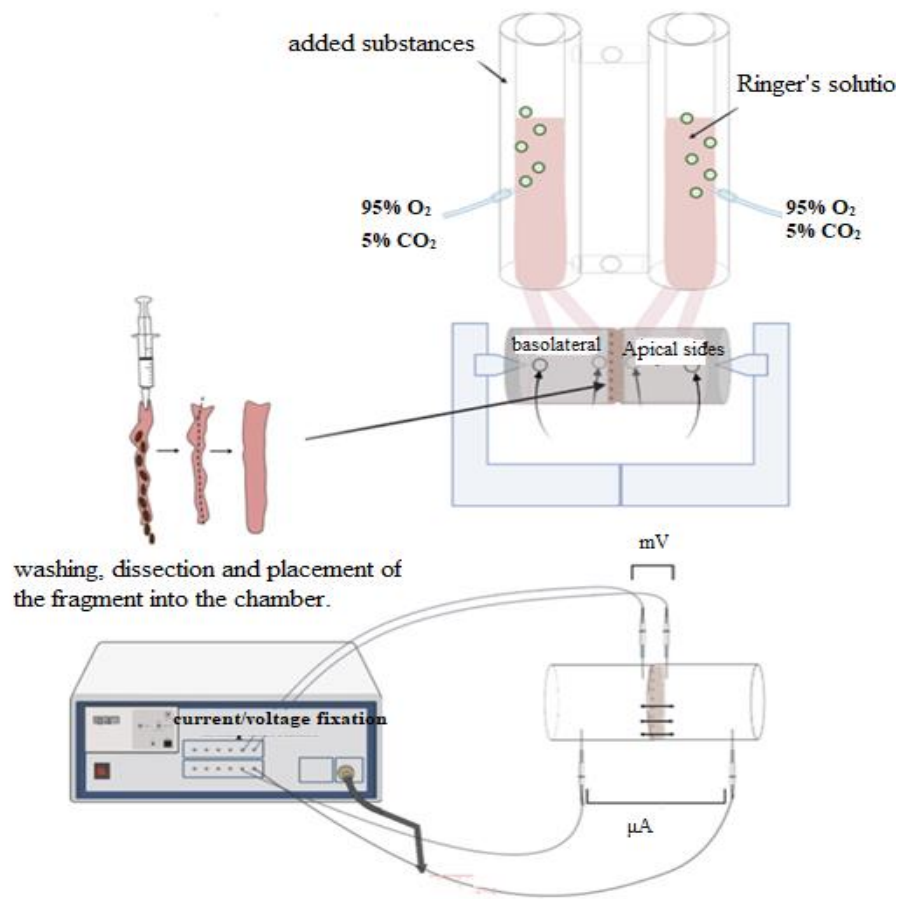


Figure (2-2). Ussing's chamber. (Blazer-Yost et al., 2022)

2.3. Study of the paracellular permeability of Peyer's patches in rat small intestine using sodium fluorescein

Sodium fluorescein (376 Da) was used to study the paracellular permeability of the Peyer's patches epithelium. 50 µl of solution containing sodium fluorescein (1 mmol concentration) was introduced into the Ussing chamber from the apical side. The final concentration of sodium fluorescein in the chamber was 100 µmol. After 60 minutes, the solution was collected from the basolateral side to determine the concentration of sodium fluorescein that had diffused through the tissue. A solution with a fluorescein concentration of 1 mmol was used to construct a standard curve. Sodium fluorescein concentration in samples was determined using a tablet and a high-resolution laser scanner Typhoon FLA 9500 (GE, USA). The excitation and absorption wavelengths of the laser were 473 and 510 nm, respectively. The obtained data were analyzed using ImageJ software. The value of permeability coefficient (Papp) was calculated by the following formula: **$P_{app} = (dQ / dt) / (A \cdot C_0)$** ,

where dQ / dt is the concentration of sodium fluorescein in the solution from the serosal side after 60 minutes of incubation (mol/s); A is the area of the tissue section under study (0.13 cm²); C₀ is the concentration of sodium fluorescein in the solution from the mucosal side at the initial moment of time (mol/l); Taking into account the ratio 1 liter = 1000 cm³, the dimension of permeability is expressed in cm/s.

2.4. Analysis of the status of goblet cells in rat small intestine by light microscopy

To assess changes in the functional state of the epithelium in the area of Peyer's patches, in particular - goblet cells, intestinal material was fixed in

buffered 10% formalin (24 h, 22°C) with subsequent prolonged washing from the fixative first in running water and then in phosphate buffer (PBS) with Ca^{2+} and Mg^{2+} . The samples were passed through a series of alcohols of increasing concentration for dehydration and encapsulated in paraffin according to standard methods.

Slices obtained using a Leica RM2265 microtome (Germany), 5 μm thick, were placed in a Sakura (Japan) water bath with distilled water heated to 38 °C for straightening and then mounted on slides before being left overnight in a thermostat at 37 °C for final stabilization of the slices on glass.

The next day, a xylene dewaxing procedure was performed (2 x 5 min; 22°C). Xylene was removed by repeated incubation in absolute alcohol and passing through alcohols of descending concentration (2 x 5 min; 22°C). The preparations were then washed in distilled water (2 x 5 min; 22°C).

Trichrome staining of preparations was used to increase the contrast of microscopic elements in cells and tissues in order to facilitate their differentiation. To detect goblet cells we used the method of staining with Alcian blue, which is able to form ionic bonds with polyanions of acidic mucopolysaccharides. A 1% solution of the dye prepared according to the standard method stains the secretion of goblet cells in bright turquoise-blue colour. In addition, the formed pigment is insoluble, which facilitates further work with the sample without undesirable spreading of the dye on the preparation.

After staining with Alcian blue for 3 minutes, the preparation was stained with a combination of eosin and Ehrlich's hematoxylin. Hematoxylin (5 min; 22°C) stains basophilic structures purple, and eosin (0.5% water-alcohol

solution, 5 min) stains cytoplasm and connective tissue structures in various shades of bright red.

This was followed by wiring in alcohols of ascending concentration 70%, 80% and 96%, isopropanol (5 min; 22°C), clarification of sections in two changes of xylene (5 min; 22°C) and encapsulation of preparations in balsam and coverslips. The coverslips were mounted on the sections using Corbit-Balsam (Hecht, Germany). The histological structure of the tissue was assessed using an inverted light microscope Leica DMI 6000 (Leica, Germany). Morphometric analysis of images was performed using ImageJ software.

2.5. Statistical processing of the obtained results

Statistical processing of the data was performed in GraphPad Prism v8 programme (Graphpad Software, USA). Two-factor analysis of variance was used to process electrophysiological indices of the colon with normal distribution. For analysing paracellular permeability values in jejunum and colon, one-factor analysis of variance was used. 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 $\pm$ standard error of the mean.

CHAPTER THREE

Results and Discussion

3.1. Results

3.1.1. Changes in electrophysiological parameters of rat Peyer's patches under acetylcholine action

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 μ A, which corresponds to the data obtained earlier.

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 (Figure 3-1A). 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. 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 (Figure 3-1B).

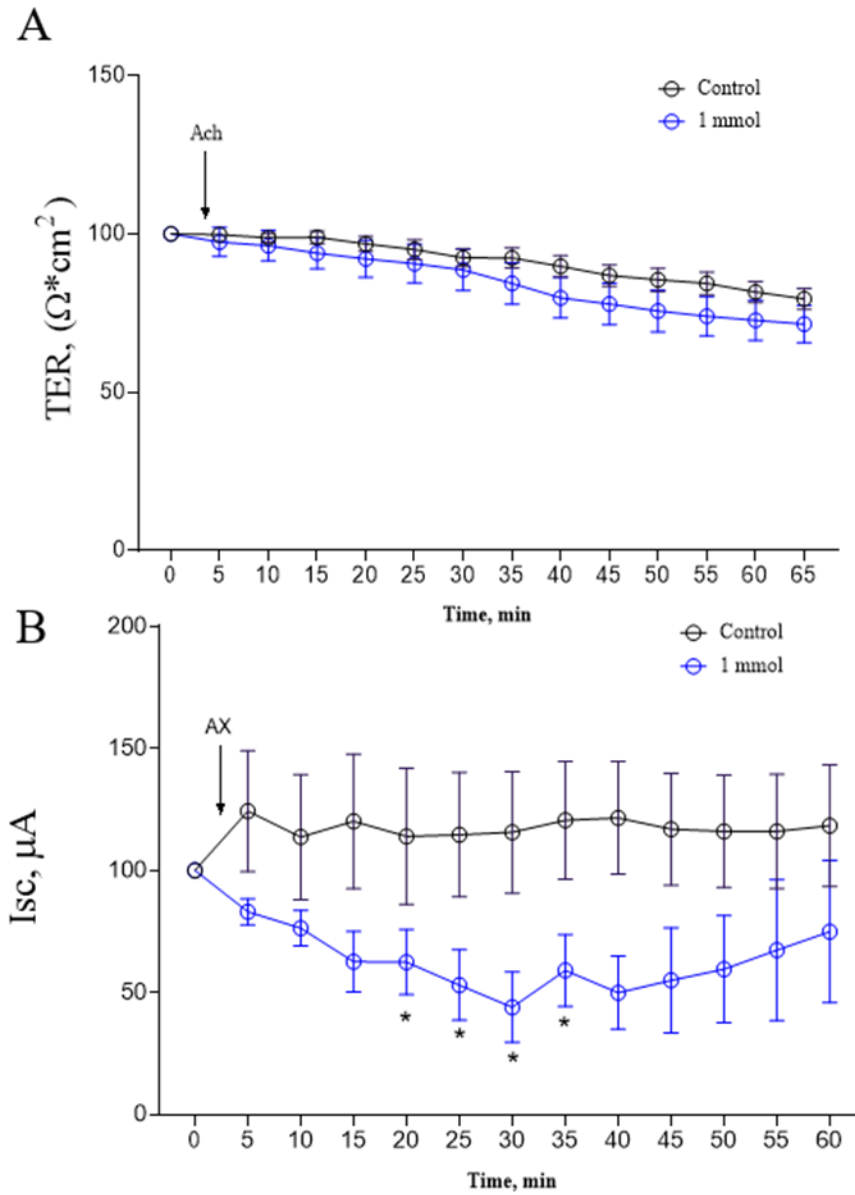


Figure (3-1) Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 1 Mmol

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%.

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 3-2A). This effect was observed for 25 minutes of incubation, and then a return to initial values is 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 (Figure 3-2B).

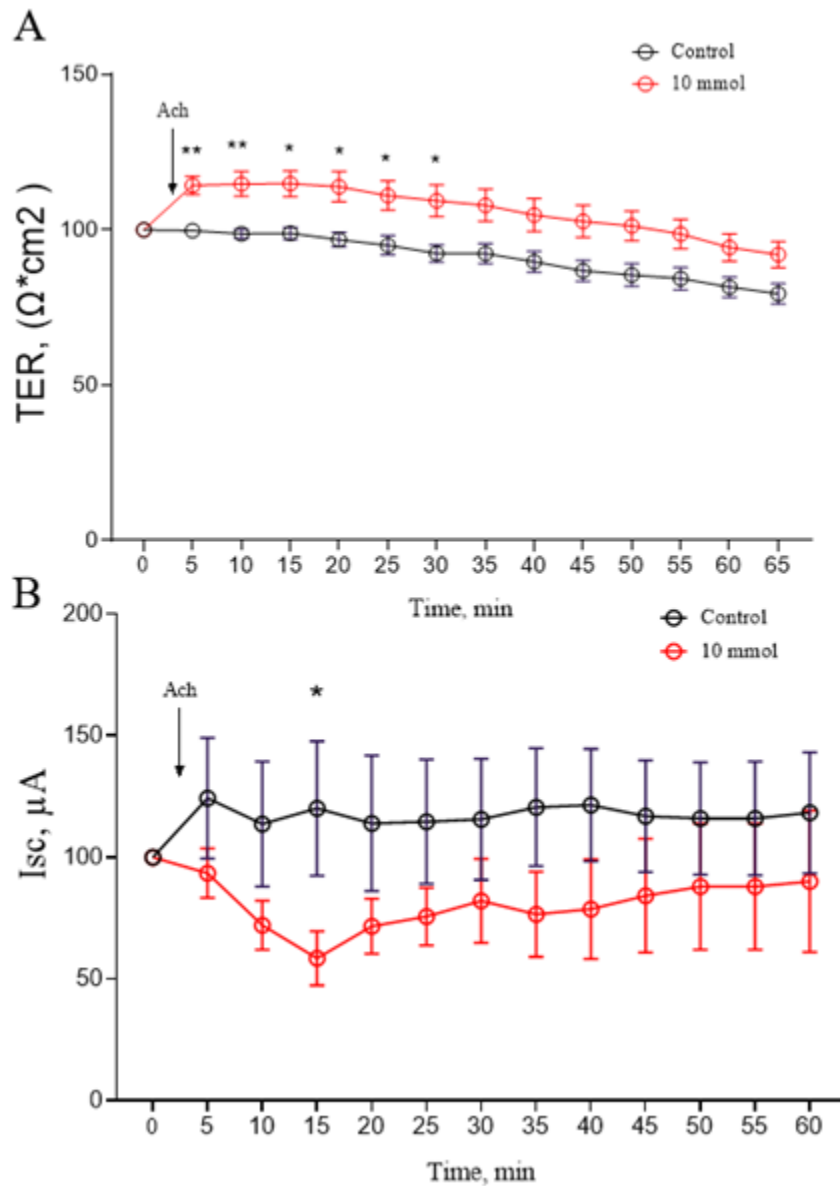


Figure (3-2) Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 10 mmol

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%.

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 (Figure 3-3A). 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) (Figure 3-3B).

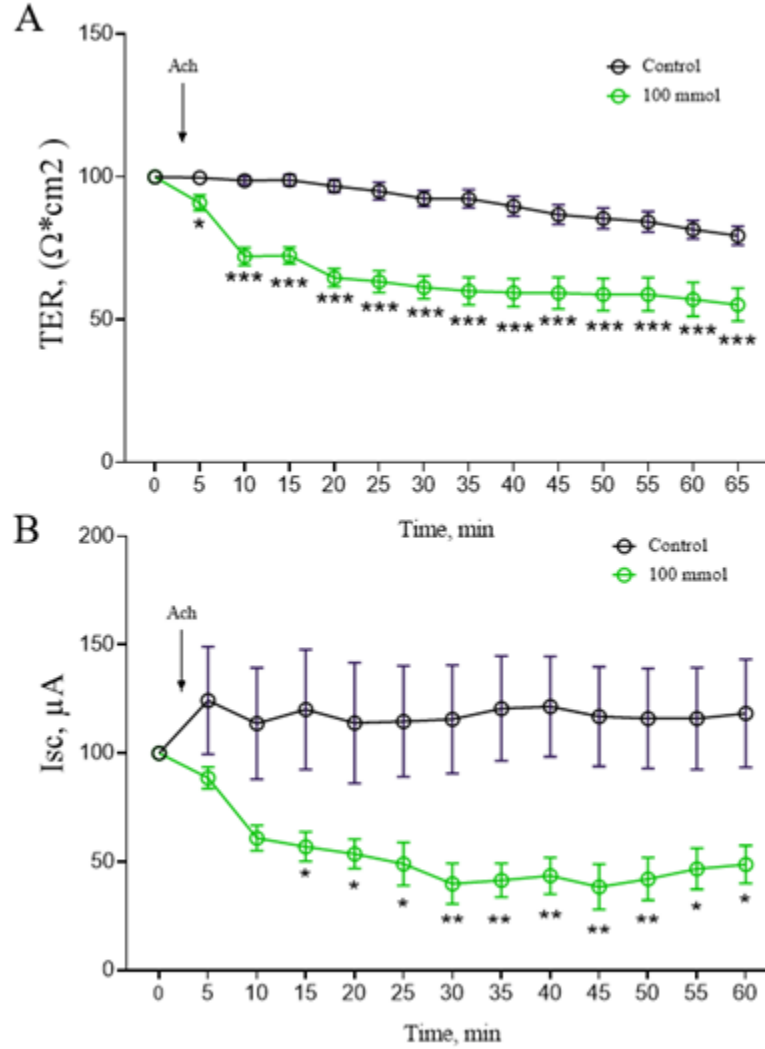


Figure (3-3) Changes in electrophysiological characteristics of Peyer's patches upon exposure to acetylcholine (ACh) at a final concentration of 100 mmol

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%.

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 to compare 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 washes in from the mucosa and serosa sides are 5 ml. The object itself is limited to the aperture area of the Ussing chamber aperture, 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 unidirectional changes when all concentrations are applied, which confirms its inhibitory effect on active ion transport through 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 in amplitude reduction of current, but less long in time. The concentration of 100 mmol causes a significant decrease of this parameter throughout the whole incubation period.

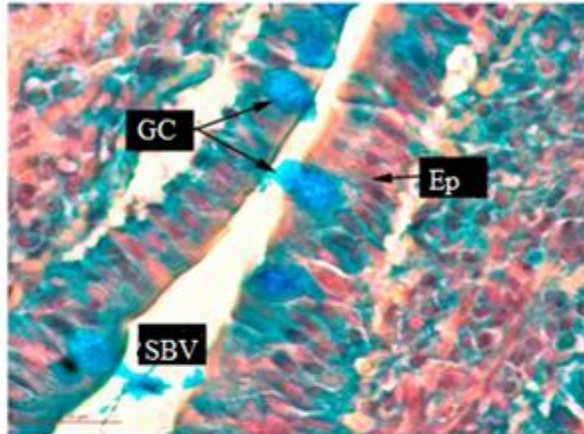
Analysing the obtained data from the point of view of subsequent pharmacological analysis, the concentration of 10 mmol should be recognized as the most promising for such an experiment. The simultaneous change in TER and short-circuit current, taking into account the processes they reflect, indicates a simultaneous change in paracellular and transcellular transport in follicle-associated epithelium of Peyer's patches of rat small intestine.

3.1.2. Effect of acetylcholine on rat Peyer's patches goblet cells during acetylcholine action

In order to verify the effect of acetylcholine at a concentration of 10 mmol, mucin secretion by goblet cells of the mucosa was studied in the current experiment. Acetylcholine is known to induce mucin secretion in the small intestine (Dolan *et al.*, 2022).

The study showed an increase in mucin secretion by goblet cells upon acetylcholine action, which allows verification of the electrophysiology data obtained (Figure 3-4).

A



B

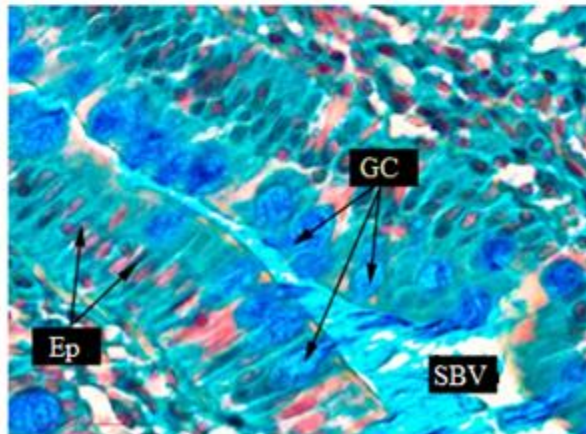


Figure (3-4) Structure of Peyer's patches epithelium in control (A) and under the action of acetylcholine at a concentration of 10 mmol (B)

GC- secreting goblet cells (staining with alcian blue and hematoxylin-eosin).

GC - secreting goblet cells (staining with alcian blue and hematoxylin-eosin), Ep- epithelium, SBV - space between intestinal villi.

Scale bar - 10 μ m

3.1.3 Changes in electrophysiological parameters of rat Peyer's patches during sequential application of mecamlamine and acetylcholine

Mecamlamine, a non-selective nAChRs antagonist, was used to elucidate a potential cholinoreceptor subtype that may be involved in altering the barrier characteristics of Peyer's patches. This cholinoreceptor subtype is widely represented in cells of the enteric nervous system and in cells of the immune system conjugated to the mucosa.

Mecamlamine was added to the Ussing chamber from the basolateral side (final concentration 1 mmol). After 30 minutes, acetylcholine was added to its final concentration in the chamber of 10 mmol. When mecamlamine was added, no changes in TER and short-circuit current were recorded in Peyer's patches preparations throughout the incubation period ($n=13$) (Figure 3-5A, B). When acetylcholine (10mmol) was administered, there was a significant increase in TER ($n=13$; $p<0.01$, two-factor analysis of variance) and a trend towards a decrease in "short circuit" current was detected ($n=13$). The pattern of response to acetylcholine, although not reproducing the values obtained in the previous series, was identical to previous results (Figure 3-4A, B). Application of mecamlamine before acetylcholine blocked the changes in TER and short-circuit current induced by acetylcholine ($n=13$) (Figure 3-5 A, B).

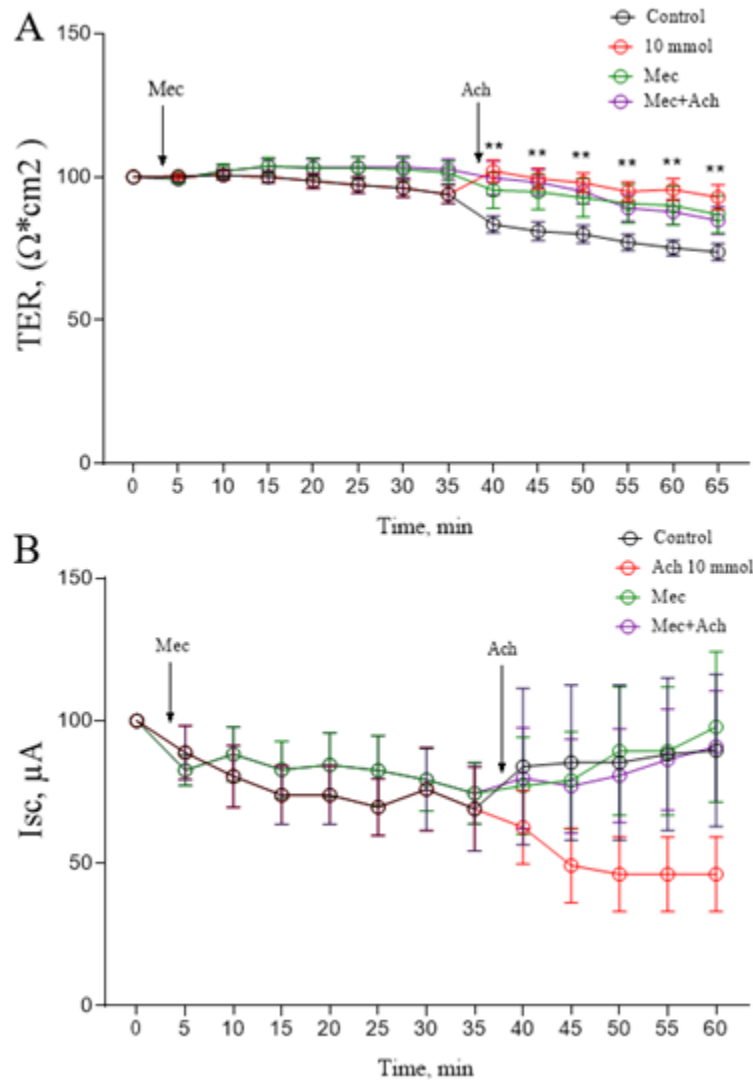


Figure (3-5) Changes in electrophysiological characteristics of Peyer's patches upon sequential application of mecamlamine and acetylcholine (ACh) at a final concentration of 10 mmol

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

B) Short-circuit current.

Arrows indicate the addition of mecamlamine and acetylcholine.

Baseline values are taken as 100%.

3.1.4. Changes in paracellular permeability of rat Peyer's patches during sequential administration of mecamlamine and acetylcholine

The initial permeability value for sodium fluorescein in Peyer's patches was $1.64 \cdot 10^{-4}$ cm/c ($n = 19$) (Figure 3-6).

The addition of acetylcholine at a concentration of 10 mmol resulted in a significant increase in paracellular permeability ($n=12$; $p<0.05$, one-way analysis of variance). Simultaneously, pre-injection of mecamlamine into the chamber was shown to prevent the increase in permeability induced by the application of acetylcholine at a concentration of 10 mmol ($n=6$; $p<0.05$, one-factor analysis of variance) (Figure 3-6).

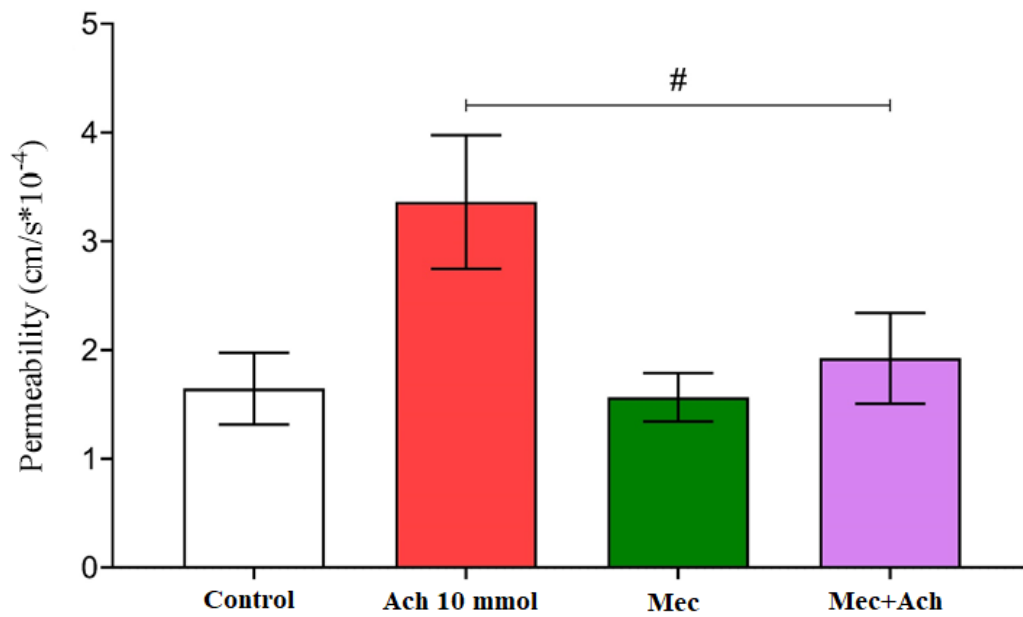


Figure (3-6) Change in paracellular permeability for sodium fluorescein in Peyer's patches upon sequential application of mecamlamine and acetylcholine (ACh) at a final concentration of 10 mmol.

$n=10-19$, # - $p < 0.05$, one-factor analysis of variance

3.2. DISCUSSION

The present study demonstrated that acetylcholine (ACh) modulates the electrophysiological and barrier properties of the follicle-associated epithelium (FAE) of Peyer's patches in the rat small intestine. The responses of transepithelial resistance (TER) and short-circuit current varied according to ACh concentration, indicating that cholinergic stimulation can regulate both epithelial barrier properties and ion transport.

At 1 mmol, ACh did not significantly alter TER, suggesting that the overall ionic barrier properties of the FAE were maintained at this concentration. However, the short-circuit current decreased following ACh administration, indicating an effect on active ion transport before a measurable alteration in epithelial resistance occurred. The subsequent recovery of the current suggests that this response was transient. These findings indicate that changes in active ion transport may occur independently of detectable changes in overall epithelial resistance. Cholinergic regulation of intestinal epithelial transport has previously been described, although the magnitude and direction of the response may depend on epithelial type and experimental conditions (Cooke et al., 2007).

Increasing the ACh concentration to 10 mmol produced a different response, with an increase in TER accompanied by a decrease in short-circuit current. The increase in TER suggests a temporary enhancement of epithelial resistance, whereas the reduction in short-circuit current indicates modification of active ion transport. The transient nature of the TER response suggests that the epithelial barrier remained capable of recovering toward its basal state. The simultaneous changes in TER and short-circuit current may therefore reflect coordinated regulation of paracellular barrier properties and transcellular ion

transport. Because this concentration produced clear changes in both parameters, 10 mmol ACh was selected for the subsequent pharmacological experiments.

At 100 mmol, ACh produced a marked and sustained decrease in TER together with a persistent reduction in short-circuit current. The decrease in TER indicates reduced epithelial resistance and suggests increased ionic permeability across the FAE. The different responses observed at 10 and 100 mmol indicate that the effect of ACh was not simply proportional to concentration. Instead, the FAE appears to respond differently according to the degree of cholinergic stimulation, potentially reflecting activation of different cellular mechanisms or differences in receptor-mediated signaling. Thus, the present findings support a concentration-dependent regulation of Peyer's patch FAE by ACh.

The reduction in short-circuit current observed at all ACh concentrations is particularly noteworthy. In conventional intestinal epithelia, cholinergic stimulation is commonly associated with enhanced ion secretion (Cooke et al., 2007). The opposite response observed in the present study may be related to the specialized structure and cellular composition of Peyer's patch FAE. This epithelium differs from conventional intestinal epithelium because it contains specialized epithelial populations and overlies lymphoid tissue. Differences in receptor distribution, ion transport mechanisms, ACh concentration, and experimental conditions may therefore contribute to the distinct electrophysiological response observed in the present study.

The increase in mucin secretion observed after exposure to 10 mmol ACh provides additional evidence that cholinergic stimulation affects epithelial function. This finding is consistent with previous evidence that ACh can stimulate mucin secretion by intestinal goblet cells (Dolan et al., 2022). Increased mucin secretion may contribute to maintenance of the epithelial surface and regulation of

interactions between luminal contents and the mucosal barrier. Therefore, the histological findings complement the electrophysiological results and indicate that the response to ACh involves not only electrical properties but also epithelial secretory activity.

The pharmacological experiments further supported the involvement of nicotinic cholinergic signaling. Mecamylamine alone produced no marked changes in TER or short-circuit current, whereas pretreatment with mecamylamine prevented the changes induced by ACh. This finding indicates that activation of nicotinic acetylcholine receptors (nAChRs) contributes to the observed electrophysiological response. Mecamylamine is a non-selective, non-competitive nAChR antagonist and is widely used to investigate nicotinic cholinergic mechanisms (Bacher et al., 2009; Nickell et al., 2013). However, because it does not selectively identify individual nAChR subtypes, the present findings cannot determine which specific receptor subtype mediates the response. Additional muscarinic or receptor-independent mechanisms also cannot be completely excluded.

The permeability experiments provided further evidence that ACh modifies the epithelial barrier. Exposure to 10 mmol ACh increased the paracellular permeability of Peyer's patch FAE to sodium fluorescein, whereas mecamylamine pretreatment prevented this increase. This finding indicates that cholinergic stimulation can modify the passage of small molecular tracers across the epithelial barrier. It is consistent with previous work by Keita et al. (2010), who reported that carbachol increased macromolecular and bacterial passage through Peyer's patch FAE, supporting a role for cholinergic signaling in the regulation of epithelial permeability. Differences between the present study and

previous work may be related to the agonist used, tracer characteristics, experimental preparation, and epithelial pathways examined.

The changes in TER and sodium fluorescein permeability were not identical, which can be explained by the different properties measured by the two methods. TER primarily reflects ionic conductance across the epithelial barrier, whereas sodium fluorescein permeability reflects the passage of a small molecular tracer. Therefore, these parameters do not necessarily change in parallel and may reflect different components of epithelial barrier regulation. The combined findings suggest that ACh can modulate several aspects of the FAE barrier rather than producing a uniform change in epithelial permeability.

The ability of mecamylamine to prevent the ACh-induced increase in sodium fluorescein permeability further supports the involvement of nicotinic signaling in barrier regulation. The lack of a marked effect of mecamylamine alone suggests that basal barrier function was not substantially altered by blockade of these receptors under the experimental conditions. Rather, nAChR-dependent signaling appears to become particularly evident during ACh stimulation.

Overall, the present findings demonstrate that ACh regulates several functional properties of Peyer's patch FAE, including epithelial resistance, active ion transport, permeability, and mucin secretion. The concentration-dependent changes in TER, the reduction in short-circuit current, and the increase in sodium fluorescein permeability indicate that cholinergic stimulation can influence both epithelial transport and barrier function. The prevention of these responses by mecamylamine further supports the involvement of nAChR-dependent signaling.

Taken together, these findings provide additional evidence that cholinergic signaling contributes to the regulation of the specialized epithelial

barrier of Peyer's patches. The combined assessment of electrophysiological parameters, sodium fluorescein permeability, histological changes, and pharmacological blockade provides an integrated view of the epithelial response to ACh. However, the specific nAChR subtype and molecular mechanisms responsible for these effects remain to be determined. Further studies examining tight-junction proteins, particularly ZO-1, occludin, and claudins, together with selective receptor antagonists and molecular approaches, may clarify the mechanisms underlying cholinergic regulation of Peyer's patch epithelial barrier function.

4.1 CONCLUSION

1. Acetylcholine modulated the electrophysiological properties of the follicle-associated epithelium of Peyer's patches in a concentration-dependent manner, affecting both transepithelial resistance and short-circuit current, and indicating alterations in paracellular and transcellular ion transport.

2. Mecamylamine alone did not significantly affect the electrophysiological properties of Peyer's patch epithelium; however, its administration prior to acetylcholine prevented the acetylcholine-induced changes in transepithelial resistance and short-circuit current, supporting the involvement of cholinergic receptors in this response.

3. Acetylcholine increased paracellular permeability to sodium fluorescein, whereas pretreatment with mecamylamine prevented this increase, indicating that cholinergic signaling contributes to the regulation of epithelial barrier permeability in Peyer's patches.

5.1 Recommendations

1. Western blot analysis should be performed to investigate the expression of tight junction proteins, such as ZO-1, occludin, and claudins, in order to clarify the molecular mechanisms underlying the changes in epithelial barrier function induced by acetylcholine.
2. Further studies should investigate the specific nicotinic acetylcholine receptor subtypes involved in the regulation of the follicle-associated epithelium, particularly $\alpha 7$ -nAChR, using selective pharmacological antagonists or molecular approaches to better define the cholinergic pathway involved in barrier regulation.
3. Future studies should combine electrophysiological measurements with molecular and morphological analyses to further clarify the relationship between changes in TER, short-circuit current, paracellular permeability, and epithelial structure following acetylcholine exposure. This may provide a more comprehensive understanding of cholinergic regulation of the Peyer's patch epithelial barrier.

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الخلاصة

يقع باير هي تراكيب متخصصة في الغشاء المخاطي المعوي. يخضع الحاجز الظهاري لبقع باير في الأمعاء الدقيقة للجرذان لتغيرات ديناميكية تحت تأثير عوامل مختلفة. تلعب هذه التغيرات دورًا حاسمًا في وظيفة الأمعاء.

دراسة وظائف الحاجز للظهارة المرتبطة بالجربيات في بقع باير في الأمعاء الدقيقة للجرذان تحت تأثير الأسيتيل كولين والتحليل الدوائي لهذه التأثيرات هي مشكلة لم يتم دراستها بشكل كافٍ حتى الآن. يمكن تحديد ذلك باستخدام التحليل الدوائي، الذي يمثل استخدام الميكاميلامين. وبالتالي، نظرًا لوجود أسيتيل كولين بشكل غير محدد في الأنسجة ولم يكن من الممكن تحديد نشاطه في تجربتنا، فقد تم اخذ تركيزات مختلفة (1 ملليمول، 10 ملليمول -100 ملليمول) لمعرفة التركيز المناسب من الأسيتيل كولين، إذ هدفت هذه الدراسة إلى التحقق من التغيرات التي تطرأ على خصائص الحاجز للظهارة المرتبطة بالجربيات في بقع باير في الأمعاء الدقيقة للجرذان تحت تأثير الأسيتيل كولين ومثبط مستقبلات الكولين ن-ميكاميلامين.

أجريت هذه الدراسة في قسم الفسيولوجيا العامة، كلية العلوم، جامعة سانت بطرسبرغ الحكومية، بالتعاون مع مركز الموارد "تطوير التقنيات الجزيئية والخلوية" التابع لجامعة سانت بطرسبرغ الحكومية، في مدينة سانت بطرسبرغ، خلال الفترة من أبريل 2022 حتى نهاية ديسمبر 2022؛ وبلغ العدد الإجمالي للعينات ($n=54$). كان المعيار الرئيسي لاختيار جزء من النسيج يحتوي على بقع باير للدراسة هو أن يكون قطره 4 مم² على الأقل.

تم وضع بقع باير بعد حضنها في الغرفة في محلول الفورمالين لغرض الفحص النسيجي باستخدام صبغة الألسيان الزرقاء، وتم تقدير الخصائص الكهربائية الفسيولوجية بواسطة غرفة أوسينج، بينما تم استخدام فلوريسئين الصوديوم (376 دالتون) لدراسة نفاذية الخلايا الظهارية لبقع باير.

أظهرت نتائج المعايير الكهربائية الفسيولوجية لبقع باير في الجرذان والتي شملت مقاومة الظهارة عبر الخلايا (TER) وتيار "الماس الكهربائي" في مستحضرات بقع باير أن إضافة الأسيتيل كولين بتركيز 1 ملي مول) لم يكن له أي تأثير على مقاومة الظهارة عبر الخلايا (TER) ونقص معنوي في تيار "الماس

الكهربائي"، بينما أدت زيادة تركيز الأستيل كولين بتركيز (10 ملي مول) إلى تغيير كل من المعايير الكهربائية الفسيولوجية. اذ لوحظ زيادة معنوية في (المقاومة عبر الظهارة) TER وانخفاض في تيار "الماس الكهربائي". من ناحية أخرى، أدت إضافة السيثيل كولين بتركيز (100 ملي مول) إلى انخفاض في كل من المعايير الكهربائية الفسيولوجية (TER وتيار "الماس الكهربائي")

من اجل التحقق من فعالية الأستيل كولين عند تركيز (10 ملي مول)، تم دراسة إفراز المخاط بواسطة الخلايا الكأسية للغشاء المخاطي. ومن المعروف أن الأستيل كولين يُسبب إفراز المخاط في الأمعاء الدقيقة. اذ لوحظ زيادة في إفراز المخاط من الخلايا الكأسية تحت تأثير الأستيل كولين، مما يسمح بالتحقق من البيانات الكهربائية الفسيولوجية المُحصَّلة. عند استخدام الميكاميلامين تحت تأثير الأستيل كولين، لا تتغير قيمة الميكاميلامين، أي التيار الكهربائي. مع الاستخدام المتتالي للميكاميلامين والأستيل كولين، لوحظ تثبيط تأثير الأستيل كولين.

في مجموعة السيطرة كانت قيم النفاذية طبيعية. يؤدي استخدام الأستيل كولين إلى زيادة كبيرة في النفاذية بين الخلايا. لا يسبب الميكاميلامين أي تغيير في النفاذية بين الخلايا. يؤدي استخدام الميكاميلامين مع الأستيل كولين إلى منع عمل الأستيل كولين.

استنادًا إلى نتائجنا، أثبتنا أن مانع مستقبلات N-كولينية الغير انتقائي الميكاميلامين يمنع التغيرات في البيانات الإليكتروفزيولوجية الحيوية، وكذلك التغيرات في انتشار فلوريسين الصوديوم في بقع باير. وبالنظر إلى أن مستقبلات N-كولينية في الغشاء المخاطي المعوي تقع في الخلايا المناعية، فإن هذا يشير إلى مشاركة هذه الخلايا في تنظيم خصائص الحاجز في بقع باير تحت التأثير الكوليني. هذا النوع من التنظيم ممكن بسبب إفراز الخلايا المناعية للسيتوكينات، التي تؤثر بشكل مباشر على الاتصالات بين الخلايا في الظهارة.

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التغيرات في الحاجز الظهاري في الظهارة المرتبطة بالجريبات في بقع باير في الأمعاء الدقيقة للجرذان

ملخص أطروحة الدكتوراه من جامعة سانت بطرسبرغ الحكومية - روسيا
الاتحادية مقدمة إلى مجلس كلية العلوم - جامعة القادسية
وهي جزء من متطلبات معادلة شهادة الاسبيرانتورا (الدكتوراه)

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