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MODULATION OF THE CONTRACTILE ACTIVITY IN THE SMOOTH MUSCLES OF THE COLON BY BIOLOGICALLY ACTIVE SUBSTANCES

The large intestine motility ensures of implementation the reservoir function, mixing of chyme, moving it into the rectum and other functions of the gastrointestinal tract. Violation of colon motility leads to pathophysiological manifestations of many diseases and pathologies of the gastrointestinal tract. Very important issue in the modern biology is possibility for correction of the tone and contractile activity in the smooth muscle cells of the large intestine.

Biologically active substances (NH₄Cl, calcium channel blocker flonidone, potassium channel activator flokalin, compound "C") modulated of the frequency and amplitude of phase components and spontaneous motor activity in smooth muscles with different frequency and intensity. It was established that the level of extracellular and intracellular pH, functioning of calcium and potassium channels of plasmatic membrane play an important role in the dynamics of motor activity of smooth sigmoid myocytes.

Keywords: myocytes; sigmoid colon; pH; flokalin; flonidone, compound "C".

Formulation of the problem. Analysis of recent research and publications. Very important is the realization of secretory, motor and absorption functions of all parts in the gastrointestinal tract for the full assimilation of nutrients.

Smooth muscles cells provided the contractile activity in the large intestine in a human. This contractile activity ensures of reservoir function realization, mixing of the chyme during digestion of nutrients and propulsion it into the rectum with subsequent removal from the body [1, 2].

The regulation of motor function occurs in the large intestine due to the complex of central and local factors, their combined effects on the intestine cells, among which nervous and humoral influences can be distinguished. All of these factors are regulators and modulators of contractile and coordinated activity in the smooth myocytes of muscular circular and longitudinal layers in the large intestine [2, 3].

Colon motility disorders lead to pathophysiological manifestations for many diseases and pathologies in the gastrointestinal tract, such as constipation, irritable bowel syndrome, diverticulosis, tumour transformations, etc. [4-7]. This is colon motility dysfunctions can be result of disorders in coordinated activity of central and autonomic nervous system, humoral motility regulation of the gastrointestinal tract, blood circulation deterioration in the submucosa and muscular layer of the intestine, dystrophy of neurons in the intermuscular neuron plexus, etc. [8-10]. Motility disorders caused by slowing down of intestine contractile activity in the digestive tract can be led to secondary dysfunctional changes in their activity. The main of them are the dysfunctional disorders of cavity, wall and membrane digestion and absorption processes, as well as disruption of homeostasis in the intestinal microbiocenosis with the development of dysbacteriosis [11, 12]. In the intestine changes internal environment composition, which intensifies the already existing disorders of digestive processes arising against this background. This leads to damage of the epithelium, the development of an inflammatory process, which marks the transition from functional disorders to beginning of a disease with a real pathological and morphological basis [13, 14].

Thus, our investigation was dedicated to study of the peculiarities of the influence by potential endo- and exogenous correctors on the intestinal motor activity as one of the keys that lead

to the high level their functionality. This investigation has an actual scientific and practical significance for modern biological science.

The purpose of the study. To study the effect of several biologically active substances on the motor activity dynamic of smooth muscle preparations in a human's sigmoid colon.

Object and research methods. This work is a fragment of the chair's scientific research work "Features of somatic, visceral and sensory systems in trained athletes at different stages of training" (state registration № 0116U001632).

We studied of smooth muscle cell contractile activity dynamic on the muscle preparations of the sigmoid colon in the mature rats. Muscle strips (2-3 mm long, 0.5-0.7 mm thick) were extracted from the longitudinal muscle layer of the sigmoid colon under a binocular microscope MBS-9 to prepare of a preparation with muscle strips, which we used in our further studies. The muscle strip has placed in a flow chamber and perfused with Krebs solution in the following composition (mmol/l): Na – 140,3; K – 5,4; Mg – 1,1; Ca – 2,5; Cl – 149,1; Tris – 10,0 (manufacturer - "Serva", Germany). The pH of solution was adjusted to 7.4; the temperature was maintained at the level of 33°C with help of automatic thermostating.

Effects of the selected experimental substances investigated by direct addition them to the perfusion solution.

The myographic method was used to study of the contractile activity dynamic in intestinal preparations. The muscle strip preparation has stretched to L_{max} , then recorded the force of contraction in the isometric mode, which carried out with help of a 6MX-1C mechanotron (according to the bridge scheme). These contractions were recorded on the N-3031 recorder, and at the same time, the contractile activity of the intestinal preparation was observed on the monitor of the C1-83 oscilloscope. The glass hook was used to connect biological and measuring systems. It was brought under the test area of the muscle preparation and connected to the mechanotron rod. The mechanotron was fixed in the PM-1M three-coordinate manipulator. The initial stretch of the muscle strip was set by vertical movement of the mechanotron.

In our investigations, we have been studying the effects of NH_4Cl solutions and the synthesized substance "C" and its separate components - the calcium channel blocker - flolidone and the potassium channel activator - flokalin on the contractile activity of intestinal smooth muscle cells. This compound was synthesized in the Organic Chemistry Institute of the Ukrainian National Academy of Sciences by supervision of prof. Yagupolsky L.M.

All test's substances has introduced into the perfusion streams at a concentration of 10^{-5} - 10^{-6} mol/l. NH_4Cl solution was used to alkalize of the Krebs solution, which perfused an isolated muscle strip in a flow chamber.

The obtained data were subjected to statistical processing using the Student's t-test, and standard application programs for statistical processing of materials also.

The experiments were conducted in accordance with the international principles of the Helsinki Declaration "Humane Treatment of Animals" (2000) and "Common Ethical Principles of Experiments on Animals" approved by the First National Congress of Bioethics (Kyiv, 2001).

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Research results and their discussion. Ours researches has shown fluctuations in the muscle tone of smooth muscle cells in the intact specimens of isolated muscle strips that was extracted from the sigmoid colon. Expressive tonic activity was recorded in 59.5% (in 22 muscle preparations out of 37 studied). The spontaneous activity was observed in the intestine smooth muscle cells in 40.5% (15 cases). (Fig. 1). Sometimes, myocytes contractile activity had clearly defined phasic components, sometimes it arose against the background of increased muscle tone.

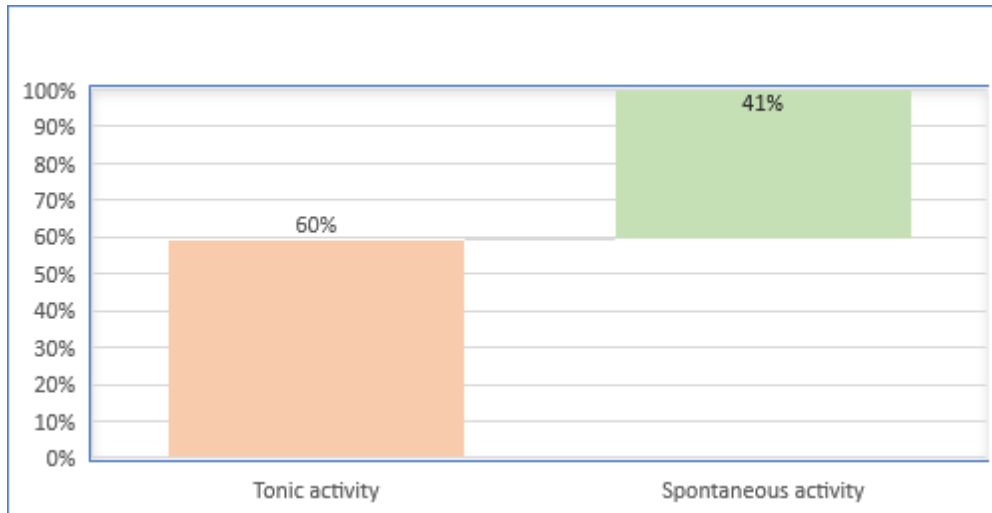


Fig. 1. Muscle activity recorded on intact samples in isolated muscle strips of smooth muscle cells extracted from the sigmoid colon.

The amplitude of contractions was 375.74 ± 97 mg in the isolated preparations of the intestinal muscle wall in the initial state. The frequency of phasic contractions was on average 0.05 ± 0.03 Hz in the isolated preparations of the intestinal smooth muscle cells that was obtained from the sigmoid colon also.

NH_4Cl solution was used to alkalize of the Krebs solution, which perfused an isolated muscle strip in a flow chamber. The NH_4Cl solution caused changes of cellular and extracellular pH in smooth muscle cells, which affected on the contractile activity of the isolated smooth muscle cell preparation. (Shown on Fig. 2).

On the figure 2 we can see, how artificial alkalinization in perfusion solution, that is washed of a muscle strip leads to the significant increase in the amplitude of phasic components the spontaneous activity in the preparations of the intestinal muscle wall up to 1200 ± 102 mg. A muscle tone increased in isolated preparations of the sigmoid colon muscle wall by an average of 475.7 ± 97 mg in the absence of an increase in the amplitude of the phasic components of spontaneous activity.

Verapamil has introduced in the perfusion solution caused an increase the amplitude of spontaneous activity and muscle tone in the smooth muscle preparations. Such contractile activity is probably associated with increased membrane permeability to calcium ions in the smooth muscle tissue of the sigmoid colon wall. This is likely possible due to the activation of the receptor apparatus in the smooth muscle cells also. Perhaps, this is the effect of the NH_4Cl molecule on the contractile activity of smooth muscle cells under alkaline conditions.



Fig. 2. Increase in the amplitude of phasic components in spontaneous motor activity in the rat's sigmoid colon under the influence of NH_4Cl ; the arrow indicates on the moment of NH_4Cl introduction into the perfusate.

In the next series of experiments, we studied the effects of ion channel modulators on the contractile activity in intestinal smooth muscle cells obtained from the sigmoid colon wall. We researched the effects of synthesized substance "C" and its separate components - the calcium channel blocker (foridone) and the potassium channel activator (flokalin) on the contractile activities of the smooth muscle cells in the investigated sigmoid colon strip. We observed the presence of the phasic and tonic muscle contraction components on intact muscle preparations of the sigmoid colon. Our experiments have shown that frequency of contraction phasic components in the sigmoid smooth muscle cells was on average 0.05 ± 0.02 Hz, and the amplitude of muscle contractions was 675.7 ± 97 mg in the initial state.

Muscle strips demonstrated decreasing of ability to spontaneous contractions after introduction of the phoridone into perfusion solution that washed the muscle preparations of the sigmoid colon intestinal wall. This was manifested to reduction of the amplitude and frequency in muscle contractions, which was accompanied by a gradual disappearance of automaticity. Foridon administration reduced muscle contractile activity in the smooth muscle preparations of the sigmoid intestinal wall, probably due to potential decrease in the concentration of Ca^{2+} ions in the cell myoplasm. Flocalin application have got a similar effect on the contractile activities of the smooth muscle cells in the investigated sigmoid colon muscle strip. The ability of a smooth muscle cells to the spontaneous activity had decreased in the investigated muscle preparations. This was evidenced by a decrease in amplitude and frequency of muscle contractions in sigmoid colon muscle strip, with the gradual disappearance of smooth muscle automaticity.

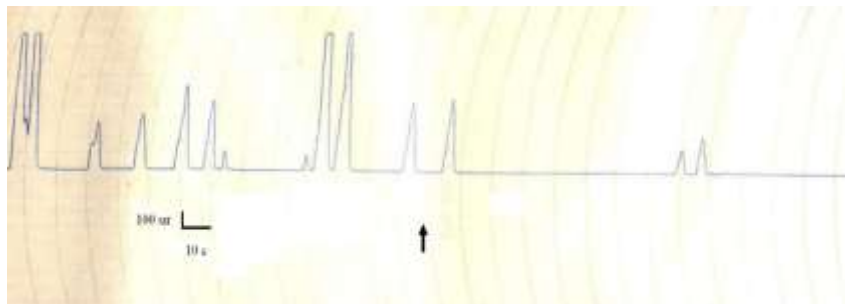


Fig. 3. The effect of substance "C" on the contractile activity of the smooth muscle preparation in the sigmoid colon; the arrow indicates on the moment of substance "C" introduction into the experimental perfusion solution.

A series of the next experiments demonstrated influence of the synthetic substance "C" on contractile activities of the investigated smooth muscle cells preparation in the sigmoid colon. The synthetic substance "C" combines in one molecule both modulators of ion channels (flocalin and foridon). The complex substance "C" effect leads to decrease in the amplitude (from 675.7 ± 97 mg to 385.6 ± 74 mg) and the frequency of phasic muscle contractions (from 0.05 ± 0.02 Hz to 0.03 ± 0.018 Hz) after introducing this substance into perfusion solution that washed smooth muscle strip in a flow chamber. This occurs more significantly (on average by 50% compared to the initial level, sometimes to complete cessation) than under the influence of flocalin or foridon alone. (Fig. 3).

Probably, substance "C" caused a significant inhibition of the calcium ion current flow through the cytoplasmic membrane into the sarcolemma of investigated smooth muscle cells in the sigmoid colon.

This is most likely associated with a myocyte's membrane low-threshold T- Ca^{2+} channels, which as usually caused periodic fluctuations of the smooth muscle cells membrane potential, that is necessary for the generation of smooth muscle spontaneous activity.

At the same time, it's activation myocyte's membrane ATP-dependent K^{+} channels are significantly increase current flow of the potassium ions through the cytoplasmic membrane into the extracellular environment from the sarcolemma of investigated smooth muscle cells.

Causally it's led to hyperpolarization of smooth myocytes plasma membrane, which ultimately leads to a decrease in the contractile activity in the sigmoid colon smooth muscle cells.

Conclusions.

1. The experimental NH_4Cl solution increased the phasic components amplitude of the smooth muscle spontaneous contractile activity due to perfusion of investigated sigmoid colon isolated muscle strip. However, muscle tone has increased if spontaneous contractile activity was absent.

2. The complex substance "C" leads to a significant reduction or cessation of the smooth muscle spontaneous contractile activities of investigated sigmoid colon isolated muscle strip.

3. Very important role plays activity of cell membrane calcium and potassium channels in the smooth muscles for the motor activity dynamics of smooth myocytes in the sigmoid colon wall. Great importance has level of extracellular and intracellular pH for the smooth muscle's contractile activity process also.

Prospects for further research. In further studies, we will have been planning to investigate the effect of selected substances on the morphofunctional organization of smooth myocytes, using electron microscopy.

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Kirichek P.V. MODULATION OF THE CONTRACTILE ACTIVITY IN THE SMOOTH MUSCLES BY BIOLOGICALLY ACTIVE SUBSTANCES

Introduction. Regulation of the contractile activity occurs due to a complex of nervous and humoral factors in the colon wall muscle tissue. Colon motility disorders lead to pathophysiological manifestations for many diseases and pathologies in the gastrointestinal tract, such as constipation, irritable bowel syndrome, diverticulosis, tumour transformations, etc. Very important signification has the possibility for tone and contractile activity potential correction in the smooth muscle cells of the human colon with help of biologically active substances, changes pH, etc.

Purpose. To study the effect of several biologically active substances on the motor activity dynamic of smooth muscle preparations in a human's sigmoid colon.

Methods. The investigations of contractile activity dynamic in the smooth muscle cells were carried out on the sigmoid colon preparations of sexually mature rats. The myographic method was used to study the dynamics of the contractile activity of intestinal preparations. All test's substances has introduced into the perfusion streams at a concentration of 10^{-5} - 10^{-6} mol/l. NH_4Cl solution was used to alkalize of the Krebs solution, which perfused an isolated muscle strip in a flow chamber. Muscle contraction force was recorded in isometric mode.

The obtained data were subjected to statistical processing using the Student's t-test, and standard application programs for statistical processing of materials also. The experiments were conducted in accordance with the international principles of the Helsinki Declaration "Humane Treatment of Animals" (2000) and "Common Ethical Principles of Experiments on Animals" approved by the First National Congress of Bioethics (Kyiv, 2001).

Results. Ours researches has shown fluctuations in the muscle tone of smooth muscle cells in the intact specimens of isolated muscle strips that was extracted from the sigmoid colon. Sometimes, myocytes contractile activity had clearly defined phasic components, sometimes it arose against the background of increased muscle tone.

The experimental synthetic substance "C" was studied. This is complex substance that combines in one molecule both modulators of ion channels (flocalin and foridon). Complex substance "C" that was administrated into perfusion solution leads to decrease in the amplitude (from 675.7 ± 97 mg to 385.6 ± 74 mg) and the frequency of phasic muscle contractions (from 0.05 ± 0.02 Hz to 0.03 ± 0.018 Hz). This is more significant (on average by 50% in relation to the initial level, sometimes to complete cessation) than under the influence of flocalin or foridon alone.

Artificial alkalization led to a significant increase in the amplitude of phasic components and spontaneous activity in the preparations of the intestinal muscle wall up to 1200 ± 102 mg.

Originality. Probably, the substance "C" caused a significant inhibition of the calcium ion current flow through the cytoplasmic membrane into the sarcolemma of investigated smooth muscle cells in the sigmoid colon. At the same time, it's activation myocyte's membrane ATP-dependent K^+ channels are significantly increase current flow of the potassium ions through the cytoplasmic membrane into the extracellular environment from the sarcolemma of investigated smooth muscle cells. Causally it's led to hyperpolarization of smooth myocytes plasma membrane, which ultimately leads to a decrease in the contractile activity in the sigmoid colon smooth muscle cells.

Conclusions.

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3. Very important role plays activity of cell membrane calcium and potassium channels in the smooth muscles for the motor activity dynamics of smooth myocytes in the sigmoid colon wall. Great importance has level of extracellular and intracellular pH for the smooth muscle's contractile activity process also.

Keywords: myocytes; sigmoid colon; pH; flocalin; foridone, compound «C».

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