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MECHANISMS OF CHANGES IN THE CONTRACTILE ACTIVITY OF SMOOTH MUSCLES OF THE GASTROINTESTINAL TRACT OF RATS UNDER PROLONGED ORAL EXPOSURE TO A MIXTURE OF POLYPROPYLENE NANO- AND MICROPARTICLES

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Background. Polypropylene ranks first in terms of world production among primary plastics. It is known that with prolonged intake into the body, polypropylene particles cause an increase in the total level of pro-inflammatory cytokines, lead to inflammation of the intestinal tissue and disruption of the intestinal barrier. In previous experiments, we showed that prolonged oral administration of a suspension of polypropylene particles causes changes in the frequency and strength of spontaneous contractions of the smooth muscles of the stomach and large intestine of rats. The aim of this work was to investigate the contractions of smooth muscles of the *antrum* and *caecum* induced by the activation of voltage-gated Ca²⁺-channels, muscarinic and nicotinic acetylcholine receptors, as well as the release of Ca²⁺ ions from intracellular stores during long-term oral consumption of polypropylene.



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Materials and Methods. The experiments were conducted on female Wistar rats. The suspension of polypropylene particles was prepared from disposable dishes according to the Roursgaard method with minor modifications. The experimental group animals received a suspension of a mixture of polypropylene nano- and microparticles (with an average hydrodynamic diameter of 1.4 μm) in drinking water (at a dose of 2.5 mg/kg daily) for 6 weeks. The contractile activity of the preparations of the circular smooth muscles of the *antrum* and *caecum* was activated by: high-potassium solution (80 mM), the agonist of muscarinic cholinergic receptors acetylcholine (10^{-5} M), the activator of the L-type voltage-gated Ca^{2+} -channels BayK 8644 (10^{-6} M), the agonist of nicotinic cholinergic receptors nicotine (10^{-4} M), and the activator of ryanodine-sensitive Ca^{2+} -channels of the sarcoplasmic reticulum caffeine (0.02 M). Contractile activity was recorded in isometric mode and analyzed by the method of Kosterin–Burdyga mechanokinetic analysis with the calculation of normalized maximum velocities of the contraction (V_{nc}) and relaxation (V_{nr}) phases.

Results. It was found that with prolonged oral intake of polypropylene particles, there is a significant increase in the amplitude of contractions of the *antrum* and *caecum*, activated by high-potassium solution and BayK 8644, due to increased influx of Ca^{2+} ions into myocytes through L-type voltage-gated Ca^{2+} -channels. Also, under these conditions, an increase in contractions of the *antrum* and *caecum*, activated by acetylcholine and nicotine, is observed due to increased cholinergic neurotransmission both at the level of enteric neurons and smooth muscle cells. With prolonged exposure to polypropylene particles, a significant increase in caffeine-induced contractions of both types of muscles is observed, caused by the release of Ca^{2+} ions from the sarcoplasmic reticulum. In the case of various contraction inducers, a significant decrease in the normalized maximum velocity of the relaxation phase was observed, likely due to a decrease in the efficiency of Ca^{2+} ion extrusion from myocytes.

Conclusions. Long-term oral administration of polypropylene particles induces a significant increase in contractile responses of the circular smooth muscles of the stomach and large intestine, caused by the pathways of electro- and pharmacomechanical excitation-contraction coupling and the release of excitatory neurotransmitters from enteric neurons, and also probably inhibits the systems of active pumping of Ca^{2+} ions from myocytes.

Keywords: mixture of nano- and microplastics, polypropylene, smooth muscles, gastrointestinal tract, voltage-gated Ca^{2+} -channels, cholinergic transmission, ryanodine receptors, contraction, mechanokinetic analysis

INTRODUCTION

Environmental pollution with plastic waste has currently reached an extremely large scale, and this critical situation has several objective reasons. Firstly, as a result of the inclusion of plastics in virtually all industrial processes, prerequisites are created for a rapid growth in the production of these substances. Thus, compared to the production of 2 million tons of plastics in 1950, by 2015, over 8300 million metric tons of primary plastic had been produced in the world, and its annual production volume is over 400 million metric tons (Geyer *et al.*, 2017; Symeonides *et al.*, 2024). Secondly, biodegradable plastics account for up to 1 % of total production, and the volumes of recycling and disposal of plastic waste are currently limited to a level of about a quarter of the amount produced in economically developed countries and less than 10 % globally (Geyer *et al.*, 2017).

Therefore, according to the Minderoo-Monaco Commission on Plastics and Human Health for 2023, the global accumulation of plastic waste in the environment is over 6 gigatons (Landrigan *et al.*, 2023).

In the environment, under the influence of physicochemical factors, plastic fragments degrade into smaller particles (destruction under the influence of mechanical impacts and freezing-thawing, photodegradation under the influence of the ultraviolet part of the solar spectrum, etc.): microplastics (particle size within 1 μm – 5 mm) and nanoplastics (particle size less than 100 nm or less than 1 μm) (Geyer *et al.*, 2017; Kadac-Czapska *et al.*, 2023; Ziani *et al.*, 2023; Symeonides *et al.*, 2024). Thus, conditions are created for the penetration into the organisms of humans and animals of a mixture of small fragments of plastics – micro- and nano-plastics (MNPs), where MNP particles primarily exert a physical effect, damaging epithelial cells of the intestine and respiratory tract, and causing activation of immune processes, induction of oxidative stress, and disruption of the microbiome (Sun *et al.*, 2024; Winiarska *et al.*, 2024; Bishop *et al.*, 2025; Cheng *et al.*, 2025). When penetrating through histohematic barriers, MNPs accumulate in tissues, concentrating especially in the intestines, liver, kidneys, and lymphoid organs (Cheng *et al.*, 2025; Dzierżyński *et al.*, 2025). The degree of toxic effect of MNPs depends on the morphology of the particles: irregularly shaped and sharp fragments cause significantly greater cell damage, particularly causing hemolysis of erythrocytes, enhanced immune response, and a 20-fold increase in the release of pro-inflammatory cytokines (Chen *et al.*, 2026). Also, serious toxic effects on the body are exerted by additional components of plastics (plasticizers, flame retardants, bisphenols, pigments, polyfluoroalkyl substances), which are mostly non-covalently bound to the polymer base (Geyer *et al.*, 2017).

MNPs enter the body through the respiratory tract and gastrointestinal tract (GIT); it is not ruled out that small amounts of nanoplastics can also enter transdermally (World Health Organization, 2022). In the case of MNP entry into the GIT, depending on their size, they can translocate into the body through mechanisms of endocytosis (up to 500 nm), uptake by intestinal macrophages (up to 1 μm), or through microfold cells in Peyer's patches (DeLoid *et al.*, 2021; World Health Organization, 2022; Agrawal *et al.*, 2024; Brouwer *et al.*, 2024). According to Senathirajah (2021), on average, human consumption of microplastics ranges from 0.1 to 5 grams per week, and under typical dietary conditions, polypropylene and polyethylene are the predominant types of MNPs detected in human biological samples (Senathirajah *et al.*, 2021).

Among non-fibrous plastics, polypropylene accounts for about 21 % of global production volume, second only to polyethylene, and ranks first (16 %) in the production of primary (non-recycled) plastics (Landrigan *et al.*, 2023). To date, a significant amount of data has been accumulated regarding the pathological effects of polypropylene nano- and microparticles on the digestive tract. Particles of plastics, particularly polypropylene, are a factor inducing inflammatory bowel diseases up to the development of colorectal cancer (Hwang *et al.*, 2019; Park *et al.*, 2023; Bruno *et al.*, 2024; Djouina *et al.*, 2026). Thus, in a model of chronic oral intake for a month in mice, a mixture of polypropylene, polyethylene terephthalate, polystyrene, and polyethylene was accompanied by changes in the transcriptome of the large intestine with a downregulation of heat shock proteins, an increased susceptibility to inflammation and oncogenesis (Djouina *et al.*, 2026). According to H. J. Gan *et al.* (2025), oral intake of polypropylene particles in mice is accompanied by an increase in the levels of pro-inflammatory markers in blood plasma (C-reactive protein, tumor necrosis factor α (TNF- α), and interleukin 10), in tissues

of the *ileum* (interleukin 1 β , TNF- α , malondialdehyde, and superoxide dismutase) and *colon* (interleukin 1 β , TNF- α , and superoxide dismutase), as well as a decrease in the expression of tight junction proteins in the *ileum* (occludin, Zo-1, and claudin-4) and *colon* (occludin and Zo-1). At the same time, manifestations of endotoxemia (increased level of bacterial lipopolysaccharide in blood plasma) are observed (Gan *et al.*, 2025). Therefore, polypropylene particles cause an increase in the overall level of pro-inflammatory cytokines, lead to inflammation of the intestinal tissue, and disrupt the intestinal barrier. Also, in our previous studies, it was shown that prolonged oral consumption of a suspension of polypropylene MNPs causes changes in the frequency and force of spontaneous contractions of smooth muscles of the stomach and large intestine of rats (Shulha *et al.*, 2026).

Until now, the effects of long-term MNP intake on the mechanisms of excitation-contraction coupling in the smooth muscles of the GIT remain understudied; therefore, the aim of this work was to investigate the contractions of smooth muscles of the *antrum* and *caecum* induced by the activation of voltage-gated Ca²⁺ channels, muscarinic and nicotinic acetylcholine receptors, as well as the release of Ca²⁺ ions from intracellular stores under long-term oral consumption of polypropylene.

MATERIALS AND METHODS

Work with laboratory animals. The experiments were performed on female Wistar rats maintained on a standard diet and under standard vivarium conditions of the Taras Shevchenko National University of Kyiv, with free access to food and water. All manipulations with rats were carried out in accordance with the International Convention for the Protection of Animals and the Law of Ukraine "On the Protection of Animals from Cruelty" (the Minutes of the Bioethics Commission of the Taras Shevchenko National University of Kyiv No. 8 dated December 26, 2024). Euthanasia of rats was carried out by hypoxia induced by carbon dioxide (CO₂).

The algorithm for loading the rat body with a suspension of polypropylene micro- and nanoparticles was chosen according to the experimental protocols described in publications (Jin *et al.*, 2019; Li *et al.*, 2020; Shulha *et al.*, 2026). In the experiments, 16 rats were used (at the beginning of the experiment, the age of the animals was 8 weeks): a control group (6 animals) and an experimental group (10 animals). Rats of the experimental group received a suspension of polypropylene particles in drinking water (at a dose of 2.5 mg/kg daily) for 6 weeks.

The preparation and characterization of the polypropylene particle aqueous suspension was described in a previous publication (Shulha *et al.*, 2026). The suspension had an average hydrodynamic diameter of 1.4 μ m; the particles were distributed in the range of 446 nm (4.4 %) – 4.02 μ m (1.5 %), and 44.2 % of them had the average diameter of 1.34 μ m (Shulha *et al.*, 2026).

Study of the contractile activity of smooth muscle preparations of the stomach and intestine. To study the contractile activity, preparations (with an average size of 2 $\times$ 10 mm) of circular smooth muscles of the *antrum* and *caecum* were used. In the experiments, Krebs solution was used (mM): 120.4 NaCl; 5.9 KCl; 15.5 NaHCO₃; 1,2 NaH₂PO₄; 1,2 MgCl₂; 2,5 CaCl₂; 11.5 glucose; the pH of the solution was 7.4.

When preparing muscle preparations, the *antrum* and *caecum*, carefully washed with Krebs solution, were freed from the mucous membranes using microinstruments

under a binocular microscope and cut in the circular direction of the arrangement of muscle fibers. Muscle preparations were stored in Krebs solution (at 4–6 °C) for no more than 24 h and used in tensiometric studies; the registration of functional activity was carried out in isometric mode. The working chamber of the tensiometric equipment was thermostated at 37°C and had an effective volume of 2 mL; the flow rate of the Krebs solution was fixed at 8 mL /min. Muscle preparations were given a passive load of 10 mN and left for at least 1 h until spontaneous contractions of a constant pattern appeared. Subsequently, contractions of the preparations were induced several times (with a washing time of 15 min) with a high-potassium solution (80 mM), which was prepared by equimolar replacement of part of Na⁺ ions with K⁺ ions.

In the study, acetylcholine (10⁻⁵ M), an activator of L-type voltage-gated Ca²⁺-channels BayK 8644 (10⁻⁶ M), nicotine (10⁻⁴ M), and an activator of ryanodine-sensitive Ca²⁺-channels of the sarcoplasmic reticulum caffeine (0.02 M) were used, all substances manufactured by “Sigma” (USA).

Mechanokinetic analysis of contractions. Analysis of contractions of the *antrum* and *caecum* preparations was carried out according to the Kosterin–Burdyga mechanokinetic analysis method (Burdyga & Kosterin, 1991). According to the method, in the coordinates:

$$\left\{ \ln \left(\frac{f_{\max} - f}{f} \right); \ln t \right\},$$

where f – is the value of the contractile force at time t and $f_{\max}$ – is the contraction amplitude). The contraction phase was defined as the mechanogram fragment from the start of the force increase to its maximum value $f_{\max}$, and the relaxation phase was the part of the spontaneous contraction from $f_{\max}$ until the force returned to the baseline level.

From the linearized plots, parameters were determined: n – the slope coefficient, and τ – the characteristic time. Subsequently, these parameters were used to calculate the normalized maximum velocities of the contraction (V_{nc}) and relaxation (V_{nr}) phases. The general formula for calculating the normalized maximum velocity is as follows:

$$V_n = \frac{(n-1)^{\frac{n-1}{n}} \cdot (n+1)^{\frac{n+1}{n}}}{4n\tau}.$$

Statistical analysis. MS Excel and OriginPro 2021 software were used for statistical analysis and calculation of mechanokinetic parameters. Samples were checked for normality using the Shapiro-Wilk test. Homogeneity of variances between the control and experimental samples was assessed using Fisher’s F-test. To determine significant differences between the mean values of the samples, a two-tailed t -test for independent groups with equal or unequal variances was employed.

The goodness of fit for data linearization with a linear function during mechanokinetic analysis was evaluated using Fisher’s test; in all cases, the coefficients of determination (R^2) were not lower than 0.96.

In all instances, results were considered significant at a probability value of $p < 0.05$. The results are presented as arithmetic mean $\pm$ standard error of the mean, where n represents the number of experiments.

RESULTS AND DISCUSSION

Modulation of voltage-gated Ca^{2+} entry into myocytes. Upon activation of the contractile activity of gastrointestinal smooth muscles, the main source of Ca^{2+} ions is the extracellular environment, from which these cations enter the myoplasm predominantly through voltage-gated Ca^{2+} -channels (VGCCs), represented by L-type channels in functioning myocytes. Application of a high-potassium solution (HPS) to muscle preparations serves as a convenient model for studying Ca^{2+} entry processes via VGCCs. Under HPS action, depolarization of the plasma membrane of myocytes occurs, triggering Ca^{2+} entry through VGCCs and, consequently, a sustained contraction (the so-called electromechanical excitation-contraction coupling model) (Kirschstein *et al.*, 2009; Ali *et al.*, 2016). Since our previous study revealed a significant increase in the amplitude of individual spontaneous contractions of the stomach and large intestine of rats that consumed a PP suspension for a long time, enhanced entry of Ca^{2+} ions into the cell myoplasm can be anticipated, particularly via the electromechanical coupling mechanism (Maselli *et al.*, 2012; Sharma & Buist, 2024; Simmonds *et al.*, 2024). Therefore, in the first stage, we investigated contractions induced by HPS application in circular smooth muscle preparations of the gastric *antrum* and *caecum* of rats.

It was found that in the case of smooth muscles of the *antrum* and *caecum* of rats that received the MNP PP suspension for 1.5 months, the amplitude of contractions activated by HPS (80 mM) increased relative to control values to an average of $181.9 \pm 13.9 \%$ and $189.1 \pm 4.0 \%$ ($p < 0.01$), respectively (**Fig. 1A**). In both cases, the tonic component of contractions remained at the control level, amounting to $123.2 \pm 12.1 \%$ ($p > 0.05$) in the *antrum* and $118.5 \pm 3.5 \%$ ($p > 0.05$) in the *caecum* (**Fig. 1B**).

Similar to spontaneous contractions (Shulha *et al.*, 2026), under PP action, the time to reach the maximum force value (t_{max}) of HPS-induced contractions increased significantly; this effect was particularly pronounced in the stomach. Thus, in the smooth muscles of the *antrum*, the t_{max} parameter reached $261.2 \pm 20.3 \%$ ($p < 0.01$), whereas in the *caecum* it averaged $140.0 \pm 5.7 \%$ relative to the control ($p < 0.05$).

Since important parameters characterizing the process of force rise and fall are the amplitude-independent normalized maximum velocities of the contraction (V_{nc}) and relaxation (V_{nr}) phases (Burdyga & Kosterin, 1991), this study analyzed these parameters in the control and under PP action. It was established that in the case of the *antrum* smooth muscles, both velocity parameters decreased significantly, reaching $51.4 \pm 6.3 \%$ and $41.8 \pm 13.7 \%$, respectively (in both cases $p < 0.01$). HPS-induced contractions of the *caecum* were characterized by V_{nc} and V_{nr} parameters at levels of $93.2 \pm 9.1 \%$ ($p > 0.05$) and $39.0 \pm 15.0 \%$ ($p < 0.01$), respectively (**Fig. 1C**).

Since we observed a significant enhancement of HPS-induced contractions under PP action, we can assume that long-term oral administration of the MNP PP suspension induces changes in smooth muscles of the stomach and large intestine, accompanied by the activation of electromechanical coupling processes. To confirm that these changes specifically involve enhanced entry of Ca^{2+} ions through VGCCs, we next compared contractions induced by the L-type VGCC activator BayK 8644 (10^{-6} M) in muscles of control animals and animals exposed to long-term PP influence. It was found that in the group of rats receiving PP, BayK 8644-induced contractions of the *antrum* and *caecum* increased to $258.3 \pm 20.35 \%$ and $341.7 \pm 24.49 \%$, respectively (in both cases $p < 0.01$). Similar to HPS-induced contractions, the tonic component did not change under the effect of PP (**Fig. 1A and B**). Consequently, long-term oral intake of PP

particles induces changes in the smooth muscle tissue of the GIT walls, in which case a significant increase in Ca^{2+} ion entry via L-type VGCCs into myocytes is observed.

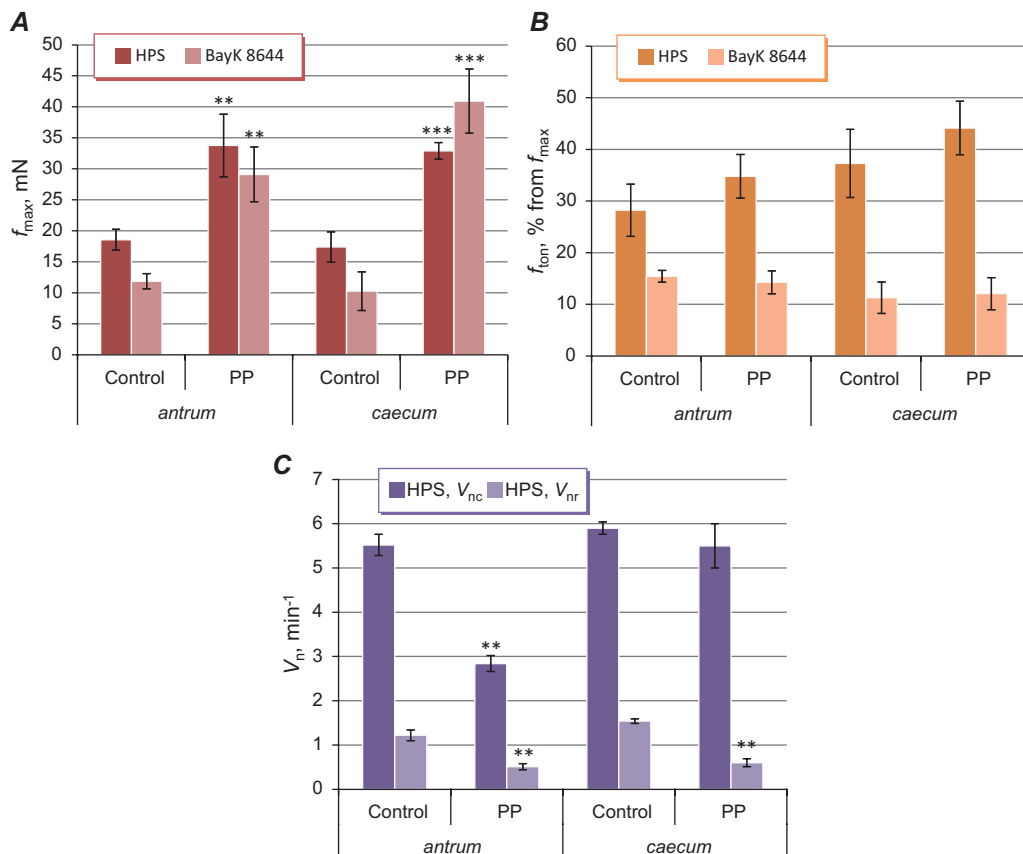


Fig. 1. Mechanokinetic parameters of contractions of the rat *antrum* and *caecum* circular smooth muscles induced by a high-potassium solution (HPS, 80 mM) and BayK 8644 (10^{-6} M) under control conditions and following long-term oral administration of polypropylene (PP) nano- and microparticle suspension: **A** – contraction amplitude (phasic component); **B** – tonic component of contractions calculated as a percentage of the amplitude; **C** – normalized maximum rates of contraction (V_{nc}) and relaxation (V_{nr}) phases of HPS-induced contractions. $n = 6-10$; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$ – significant difference relative to the control

Modulation of cholinergic neurotransmission. Acetylcholine is the primary excitatory neurotransmitter in the smooth muscles of GIT walls. It is capable of mediating the process of pharmacomechanical excitation-contraction coupling by directly activating muscarinic acetylcholine receptors in the membranes of myocytes and interstitial cells of Cajal. Furthermore, at the level of intramural nerve ganglia, it acts on nicotinic acetylcholine receptors of postganglionic neurons, triggering the release of various neurotransmitters from neurons of the intramural plexus (Igarashi-Hisayoshi *et al.*, 2023; Kohl *et al.*, 2023; Zholos *et al.*, 2024). In the next stage, we investigated the properties of acetylcholine-mediated processes in the smooth muscles of the *antrum* and *caecum* of rats subjected to long-term oral MNP PP suspension intake.

It was established that a long-term action of PP particles results in the activation of contractions of the *antrum* and *caecum* caused by acetylcholine application (10^{-5} M). Thus, the amplitude of these contractions relative to control values averaged 169.4 ± 6.8 % in the *antrum* and 186.2 ± 4.2 % in the *caecum* (in both cases ($p < 0.01$)) (Fig. 2A). As with HPS-activated contractions, under PP action, the tonic component of acetylcholine-induced contractions of both muscles did not differ from control values (Fig. 2B), while the time to reach the maximum value ($t_{\max}$) increased substantially: in the *antrum* to an average of 560.3 ± 10.8 % ($p < 0.001$), and in the *caecum* to an average of 195.5 ± 2.3 % ($p < 0.01$).

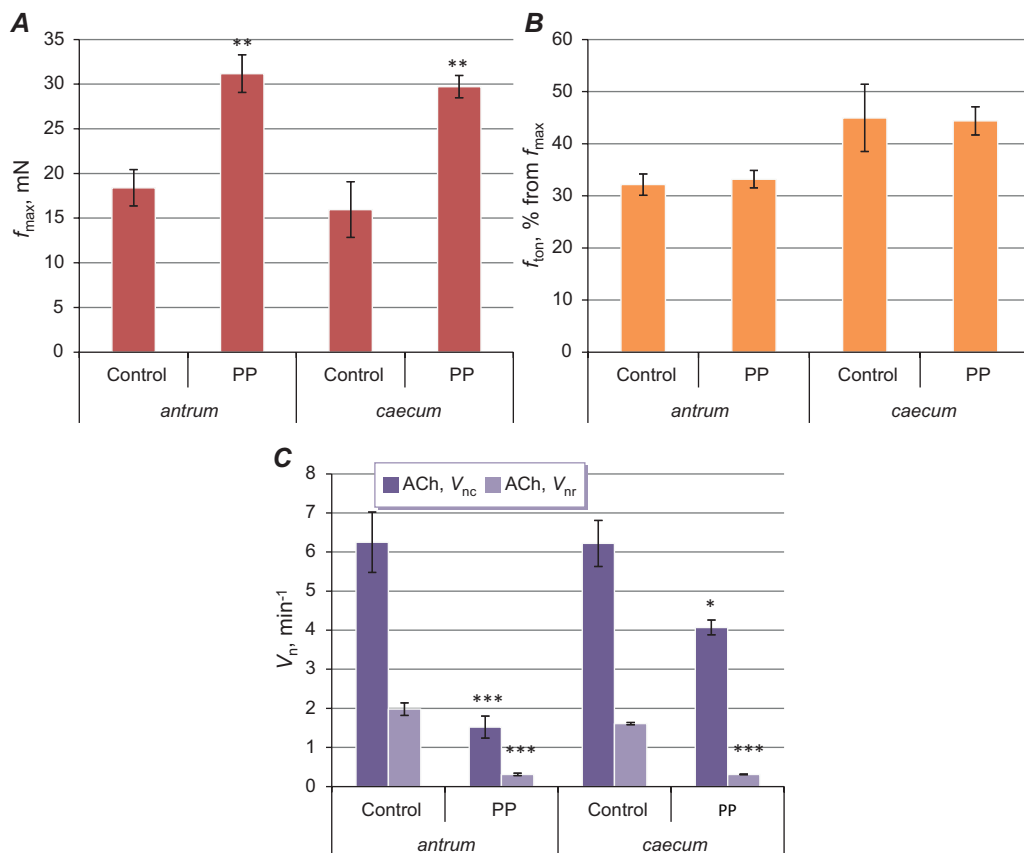


Fig. 2. Mechanokinetic parameters of contractions of the rat *antrum* and *caecum* circular smooth muscles induced by acetylcholine (ACh, 10^{-5} M) under control conditions and following long-term oral administration of polypropylene (PP) nano- and microparticle suspension: **A** – contraction amplitude (phasic component); **B** – tonic component of contractions calculated as a percentage of the amplitude; **C** – normalized maximum rates of contraction (V_{nc}) and relaxation (V_{nr}) phases of ACh-induced contractions. $n = 6-10$; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$ – significant difference relative to the control

Also, under MNP action, a significant decrease in the normalized maximum velocities of the contraction and relaxation phases was observed. Thus, V_{nc} and V_{nr} parameters in the *antrum* smooth muscles were 24.3 ± 18.4 % and 15.7 ± 9.7 %, respectively

(in both cases $p < 0.001$), and in the *caecum* smooth muscles – $65.4 \pm 4.7\%$ ($p < 0.05$) and $19.3 \pm 3.2\%$ ($p < 0.001$), respectively (**Fig. 2C**). Therefore, under long-term *in vivo* action of the PP particle suspension, an enhanced response to the parasympathetic neurotransmitter acetylcholine is observed in the smooth muscles of the digestive tract.

Next, the effects of nicotine, a nicotinic acetylcholine receptor agonist, were investigated. Nicotine application (10^{-4} M) to smooth muscle preparations in the control group elicited a biphasic response: an initial relaxation followed by a phasic contraction. Under the influence of the PP particle suspension, an increase in the amplitude of nicotine-activated contractions of the *antrum* smooth muscles (to an average of $286.2 \pm 21.3\%$, ($p < 0.01$) and the *caecum* smooth muscles (to an average of $405.7 \pm 6.1\%$, ($p < 0.001$) occurred (**Fig. 3A**). Under these conditions, the parameters of the tonic components of contractions also increased (**Fig. 3B**).

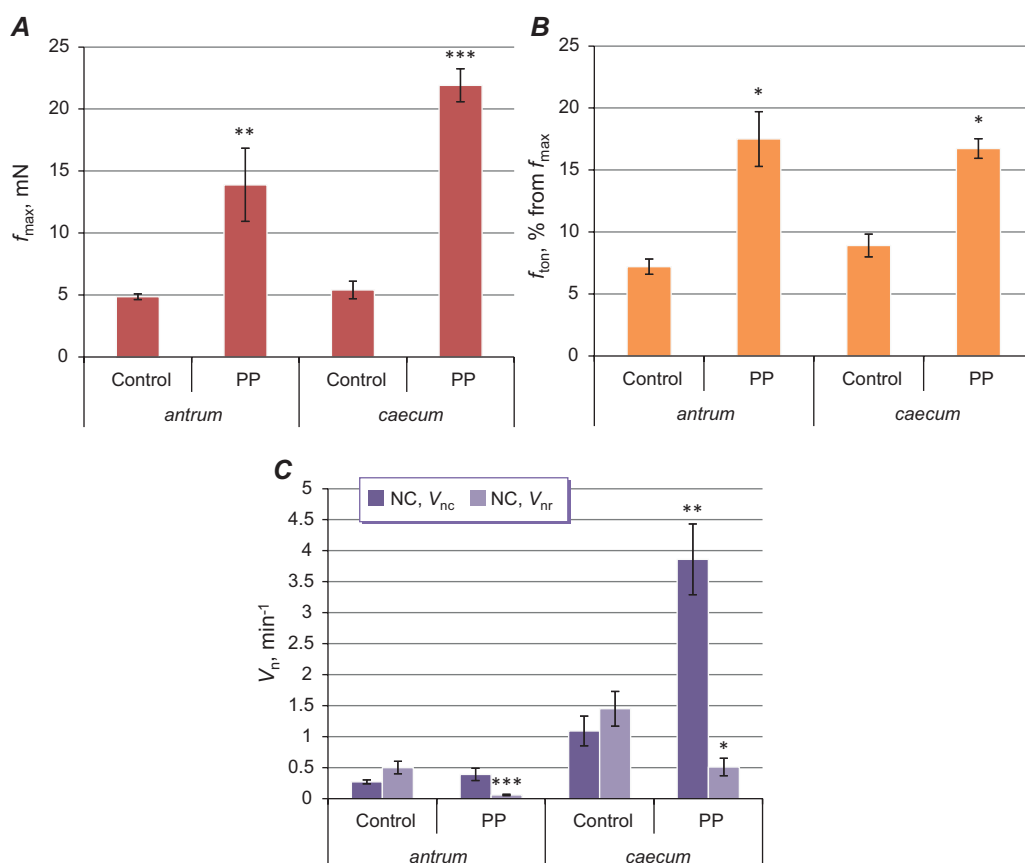


Fig. 3. Mechanokinetic parameters of contractions of the rat *antrum* and *caecum* circular smooth muscles induced by nicotine (NC, 10^{-4} M) under control conditions and following a long-term oral administration of polypropylene (PP) nano- and microparticle suspension: **A** – contraction amplitude (phasic component); **B** – tonic component of contractions calculated as a percentage of the amplitude; **C** – normalized maximum rates of contraction (V_{nc}) and relaxation (V_{nr}) phases of NC-induced contractions. $n = 6-10$; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$ – significant difference relative to the control

Interestingly, unlike HPS- and acetylcholine-induced contractions, a decrease in the $t_{\max}$ parameter was observed in the case of nicotinic contractions. In the *antrum* smooth muscles, it averaged 43.8 ± 16.3 % ($p < 0.05$), and for the *caecum* preparations, it equaled an average of 39.0 ± 28.4 % ($p < 0.05$). Furthermore, in the case of these contractions, the normalized maximum velocity parameter V_{nc} in the *antrum* muscles showed a tendency to increase (144.4 ± 25.6 %, $p > 0.05$), while in the *caecum* muscles, it increased significantly (354.1 ± 14.8 %, $p < 0.01$). The normalized maximum velocity parameter of the relaxation phase V_{nr} decreased, amounting to 11.6 ± 7.2 in the *antrum* muscles ($p < 0.01$) and an average of 35.2 ± 27.5 in the *caecum* muscles, ($p < 0.05$) (**Fig. 3C**).

Thus, during long-term *in vivo* exposure to a polypropylene particle suspension, an enhanced contribution of cholinergic regulation to gastric and colonic motility is observed in the smooth muscles of the digestive tract.

Modulation of Ca^{2+} release from the sarcoplasmic reticulum. As shown above, long-term intake of MNP PP into the organism causes an enhancement of electromechanical and pharmacomechanical excitation-contraction coupling processes (Maselli *et al.*, 2012; Sharma & Buist, 2024; Simmonds *et al.*, 2024). However, it must be taken into account that the entry of Ca^{2+} ions into the cell triggers the release of these cations from the intracellular store of the sarcoplasmic reticulum via ryanodine-sensitive Ca^{2+} channels (RyRs). This is the so-called process of Ca^{2+} -induced Ca^{2+} release (Hennig *et al.*, 2002; Leybaert & Sanderson, 2012). Intensification of this process might be one of the reasons for the enhanced contractile activity of muscles. Therefore, in the next stage, we investigated contractions of the *antrum* and *caecum* smooth muscles induced by the RyR activator caffeine (20 mM).

It was found that the smooth muscles of rats exposed to long-term *in vivo* action of the polypropylene particle suspension exhibit a significantly greater response to caffeine. Thus, an average amplitude of muscle contractions in the *antrum* and *caecum* was 553.4 ± 11.7 % ($p < 0.001$) and 400.5 ± 22.4 % ($p < 0.001$), respectively (**Fig. 4A**).

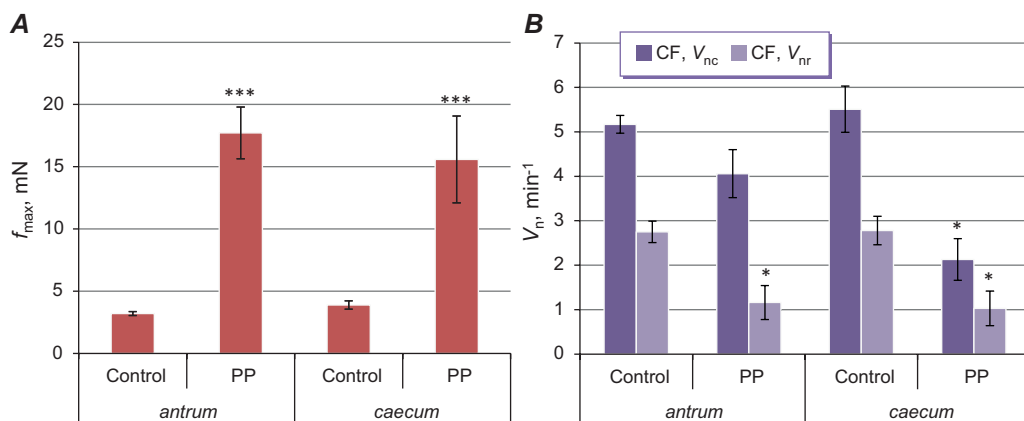


Fig. 4. Mechanokinetic parameters of contractions of the rat *antrum* and *caecum* circular smooth muscles induced by caffeine (CF, 0.02 M) under control conditions and following long-term oral administration of polypropylene (PP) nano- and microparticle suspension: **A** – contraction amplitude (phasic component); **B** – normalized maximum rates of contraction (V_{nc}) and relaxation (V_{nr}) phases of CF-induced contractions. $n = 6-10$, * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ – significant difference relative to the control

Also, under MNP action, a decrease in the normalized maximum velocities of the contraction and relaxation phases was observed. Specifically, V_{nc} and V_{nr} parameters in the *antrum* smooth muscles were 78.5 ± 13.3 % and 42.2 ± 22.8 % respectively ($p < 0.05$), and in the *caecum* smooth muscles – 38.7 ± 22.1 % and 37.1 ± 27.9 %, respectively, ($p < 0.05$) (**Fig. 4B**). Consequently, a long-term *in vivo* action of the polypropylene particle suspension in the smooth muscles of the digestive tract also induces the activation of Ca^{2+} ion release processes through RyRs, which can likewise cause a substantial increase in smooth muscle responsiveness to both depolarization and excitatory neurotransmitters of the intramural nervous system.

Our study first revealed the individual mechanisms by which the motor function of the smooth muscles of the digestive tract is impaired with prolonged daily intake of a mixture of polypropylene nanoparticles and microparticles. The results obtained may have practical implications for the development of strategies for directed normalization of gastrointestinal motility in the presence of disorders associated with prolonged oral exposure to microplastics, including inflammation, dysbacteriosis, endotoxemia, and impaired intestinal barrier function. In the future, it is promising to study the mechanisms of functioning of the smooth muscles of the gastrointestinal tract with prolonged daily intake of other most common environmental plastic pollutants, in particular, polyethylene, polyethylene terephthalate and polystyrene.

CONCLUSIONS

Regular prolonged intake of a suspension of nano- and micro-sized polypropylene particles into the body is accompanied by activation of excitation-contraction processes via electro- and pharmacomechanical coupling in the smooth muscles of the stomach and large intestine. Under these conditions, there is an increase in the influx of Ca^{2+} ions through the L-type VGCCs, a significant increase in the amplitude of acetylcholine-induced contractions, as well as contractions caused by the release of these cations through RyR from the sarcoplasmic reticulum. Also, under the action of MNP, the activation reactions of smooth muscles to nicotine application were significantly enhanced. The decrease in the normalized maximum velocity of the relaxation phase, which was observed in the case of various contraction inducers, can probably be associated with a decrease in the efficiency of extrusion of Ca^{2+} ions from myocytes under prolonged action of PP.

Thus, prolonged oral intake of PP into the body causes a violation of the contractile function of the smooth muscles of the stomach and large intestine, the mechanisms of which are the enhancement of potential- and receptor-dependent processes of the influx of Ca^{2+} ions into smooth muscle cells, as well as the inhibition of the pumping of these cations from the myoplasm.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that they have no conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: all international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [O.T.; M.S.]; methodology, [M.S.; O.T.; I.V.; A.S.; T.D.; S.B.; O.C.; V.B.; K.S.; Y.S.]; research, [M.S.; O.T.; I.V.; T.D.; S.B.; O.C.; V.B.; Y.S.]; resources, [M.S.; O.T.; I.V.; T.D.]; data processing, [M.S.; O.T.; A.C.]; writing – preparation of the original project, [M.S.; O.T.]; writing – review and editing, [M.S.; O.T.; I.V.; O.S.; T.D.; S.B.; O.C.; V.B.; K.S.; Y.S.]; visualization, [M.S.; O.T.] supervision, [O.T.]; project management, [O.T.]; funding search, [–].

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МЕХАНІЗМИ ЗМІН СКОРОЧУВАЛЬНОЇ АКТИВНОСТІ ГЛАДЕНЬКИХ М'ЯЗІВ ШЛУНКОВО-КИШКОВОГО ТРАКТУ ЩУРІВ ЗА ТРИВАЛОГО НАДХОДЖЕННЯ В ОРГАНІЗМ СУМІШІ НАНО- І МІКРОЧАСТИНОК ПОЛІПРОПІЛЕНУ

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Обґрунтування. Поліпропілен за обсягом світового виробництва серед первинних пластиків займає перше місце. Відомо, що за тривалого надходження в організм частинки поліпропілену спричиняють зростання загального рівня прозапальних цитокінів, спричиняють запалення тканини кишечника і призводять до порушення кишкового бар'єру. У попередніх дослідях ми з'ясували, що тривале пероральне вживання суспензії МНП поліпропілену спричиняє зміну частоти і сили спонтанних скорочень гладеньких м'язів шлунку і товстого кишечника щурів. Метою цієї роботи було дослідити скорочення гладеньких м'язів антрального відділу шлунку та сліпої кишки, індуковані активацією потенціалкерованих Ca^{2+} -каналів, мускаринових і нікотинових холінорецепторів, а також вивільнення іонів Ca^{2+} з внутрішньоклітинних депо, за тривалого перорального вживання поліпропілену.

Матеріали та методи. Експерименти проводили на самках щурів лінії Вістар. Суспензію частинок поліпропілену готували з одноразового посуду за методом Roursgaard із незначними модифікаціями. Тварини експериментальної групи упродовж 6 тижнів отримували суспензію суміші нано- і мікрочастинок поліпропілену

(зі середнім гідродинамічним діаметром 1,4 мкм) у питній воді (в дозі 2,5 мг/кг щодобово). Скорочувальну активність препаратів кільцевих гладеньких м'язів антрального відділу шлунку (*antrum*) і сліпої кишки (*caecum*) активували: гіперкалієвим розчином (80 мМ), агоністом мускаринових холінорецепторів ацетилхоліном (10^{-5} М), активатором потенціалкерованих Ca^{2+} -каналів L-типу BayK 8644 (10^{-6} М), агоністом нікотинових холінорецепторів ніотином (10^{-4} М) і активатором ріанодин-чутливих Ca^{2+} -каналів саркоплазматичного ретикулуру кофеїном (0,02 М). Скорочувальну активність реєстрували в ізометричному режимі й аналізували механокінетичним методом Костеріна–Бурдиги із розрахунком нормованих максимальних швидкостей фаз скорочення (V_{nc}) і розслаблення (V_{nr}).

Результати. Встановлено, що за тривалого перорального надходження частинок поліпропілену відбувається суттєве зростання амплітуди скорочень *antrum* і *caecum*, активованих гіперкалієвим розчином і BayK 8644, внаслідок посилення надходження іонів Ca^{2+} у міоцити через потенціалкеровані Ca^{2+} -канали. Також за цих умов спостерігали збільшення скорочень *antrum* і *caecum*, активованих ацетилхоліном і ніотином, через посилення холінергічної нейротрансмісії як на рівні ентеральних нейронів, так і на рівні гладеньком'язових клітин. За тривалої дії частинок поліпропілену спостерігали значне зростання кофеїн-індукованих скорочень обох типів м'язів, обумовлених вивільненням іонів Ca^{2+} зі саркоплазматичного ретикулуру. У разі різних індукторів скорочень спостерігали суттєве зменшення нормованої максимальної швидкості фази розслаблення, ймовірно, внаслідок зниження ефективності екструзії іонів Ca^{2+} з міоцитів.

Висновки. Тривале пероральне надходження частинок поліпропілену індукує значне посилення скорочувальних відповідей кільцевих гладеньких м'язів шлунку і товстого кишечника, спричинених шляхами електро- і фармакомеханічного спряження збудження-скорочення та вивільненням збуджувальних нейромедіаторів з ентеральних нейронів, а також, ймовірно, пригнічує системи активного викачування іонів Ca^{2+} з міоцитів.

Ключові слова: гладенькі м'язи, шлунково-кишковий тракт, суміш нано-і мікропластику, поліпропілен, потенціалкеровані Ca^{2+} -канали, холінергічна трансмісія, ріанодинові рецептори, скорочення, механокінетичний аналіз