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## INFLUENCE OF NANO-TiO<sub>2</sub> ON FUNCTIONING OF GASTRIC SMOOTH MUSCLES: *IN VITRO* AND *IN SILICO* STUDIES

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Nanosized materials, including titanium dioxide nanoparticles, sized under 10 nm, are systems with an excessive energy and high chemical activity, while the nanoparticles of about (1–3) nm enter the reactions with other chemical compounds practically without any activation energy which predetermines the formation of substances with new properties. The energy accumulated by these objects first of all is determined by the uncompensated nature of the bonds between surface and near-surface atoms that is a reason of superficial phenomena. Taking the abovementioned into consideration, it was interesting to study the influence of nano-titanium dioxide sized (1–3) nm and (4–8) nm on the functioning of rat gastric smooth muscles *in vitro* and *in silico*.

The tenzometric method in the isometric mode was used to demonstrate that titanium dioxide suspensions with nanoparticles, sized (4–8) and (1–3) nm, change the structure of spontaneous contraction cycles for circular stomach smooth muscles of antrum in rats with a decrease in their total efficiency (a decrease in index of contractions in Montevideo units (MU) and the index of contractions in Alexandria units (AU)). In these conditions, there was also a change in the kinetic parameters of high potassium contractions and the contractions induced by acetylcholine, the mediator of acetylcholine receptors. There was also an impairment of the processes of coordinating the velocities of contractions and relaxations, that are more expressed in the first case at the effect of titanium dioxide (1–3) nm, and in a second one – (4–8) nm. The molecular docking of titanium dioxide nanoparticle to an extracellular part of a muscarinic acetylcholine M2 type receptor demonstrated a possibility of forming the bonds with some amino acids of the site of its allosteric modulator, that impacts the affinity of this receptor to the orthosteric ligands. The binding site of titanium dioxide does not compete for binding sites

of this type of acetylcholine receptor neurotransmitter by its amino acid composition. The molecular docking of titanium dioxide to the muscarinic acetylcholine M3 type receptor showed that there are common amino acid residues for both the nanoparticle and acetylcholine with which bonds are formed in the orthosteric binding site. This suggests that at this binding site there can be a competitive relationship between titanium dioxide and acetylcholine within the site.

**Keywords:** tenzometric method, circular smooth muscles, titanium dioxide, molecular docking, muscarinic acetylcholine receptors

## INTRODUCTION

The improvement of methods of synthesizing nanostructures [1, 15, 21, 22, 25, 31] promoted a transition to high modern technologies of manufacturing nanoparticles, quantum dots, quantum well, quantum wires, etc. Actually, the constructed nanostructures are artificially created materials with preset physical and physical-chemical properties defined by their sizes. The size of particles is an active variable defining the state of the system and its reactive capability along with other thermodynamic variables [18]. One of these artificially synthesized nanomaterials is nanosized titanium dioxide ( $\text{TiO}_2$ ). Being an integral part of the technological processes in such modern industries as hydrogen energy, photovoltaics, sensorics, etc.,  $\text{TiO}_2$  is ranked among the top manufactured chemicals in economy due to its high chemical stability and activity in interactions with other chemical substances and physical factors as well as its high absorption properties. While present in several modifications, it is a rutile form in which  $\text{TiO}_2$  is a single-option white pigment, the suspension of nanoparticles of which is widely used in food industry (food additive E171).  $\text{TiO}_2$  in a nanosized form (especially 5 nm) is also systematically used in the production of tableted medications, nanomedicine, and aerosols [5, 6, 29, 40]. Regardless of active introduction of technologies of their production in both food and pharmaceutical industries, there are no models of evaluating the risk and safety standards regarding the effect of most nanostructures, including  $\text{TiO}_2$ , on molecular and cellular mechanisms of regulating the functions of the organism [32]. The results of our *in vitro* research demonstrate [33, 35] that the targets of the effect of  $\text{TiO}_2$  suspension with the size of nanoparticles ( $21 \pm 5$ ) nm may be receptor-dependent regulatory mechanisms in smooth cells of stomach, caecum, and myometrium. The *in vivo* experiments established [34] that the accumulation of  $\text{TiO}_2$  with the same size of nanoparticles in stomach smooth muscles (that was demonstrated using atomic emission spectrometry) leads to a considerable increase in frequency of their spontaneous contractions, a decrease in the duration of the contraction-relaxation cycle, a reduction in the efficiency indices for functioning of muscles (MU and AU indices of contractions).

It is known [17, 23] that the nanoparticles including  $\text{TiO}_2$  sized under 10 nm are systems with an excessive energy and high chemical activity, while nanoparticles of about (1–3) nm enter the reactions with other chemical compounds practically without any activation energy which predetermines the formation of substances with new properties. The energy accumulated by these objects is first of all determined by uncompensated nature of the bonds between surface and near-surface atoms which is a reason of superficial phenomena. Taking the abovementioned into consideration, it was interesting to study changes in the amplitude-frequency and kinetic parameters of pacemaker

activity at the effect of the suspension of TiO<sub>2</sub> with the nanoparticles sized (1–3) nm and (4–8) nm, and the contractions of stomach smooth muscles (SM) induced by high potassium Krebs solution and acetylcholine – the agonist of muscarinic cholinergic receptors, and to compare them with the results, previously obtained in the experiments [33] on investigating the impact of TiO<sub>2</sub> suspension with nanoparticles sized (21 ± 5) nm on the above-mentioned parameters. The study was also aimed at conducting molecular docking of the nanosized TiO<sub>2</sub> to the extracellular part of the muscarinic M2 type cholinergic receptor.

## MATERIALS AND METHODS

8-week-old Wistar rats of both genders were used for *in vivo* experiments. The rats were kept in standard conditions of the vivarium (room temperature of 20 ± 2 °C, relative humidity – 50–70 %, light-darkness cycle – 12:12 h). All manipulations with animals were carried out in accordance with the International Convention of animals and the Law of Ukraine “On protection of animals from cruelty”. Protocol N 2 (October 20, 2016) of the meeting of Bioethics Committee of the Educational and Scientific Centre “Institute of Biology and Medicine” Taras Shevchenko KNU. Killing of animals carried out by the injection of a lethal dose of anesthetic propofol (Sigma).

The experiments *in vitro* were conducted using isolated preparations of circular smooth muscles of rats' antrum. The abduction and registration of spontaneous contractive activity, K<sup>+</sup>-induced contractions and contractions of muscle induced by exogenous application of acetylcholine were conducted using the tencometric method in an isometric mode. The normal Krebs solution (NRS) was used in the experiments with the following concentration of components (mM): NaCl – 120.4; KCl – 5.9; NaHCO<sub>3</sub> – 15.5; NaH<sub>2</sub>PO<sub>4</sub> – 1.2; MgCl<sub>2</sub> – 1.2; CaCl<sub>2</sub> – 2.5; glucose – 11.5; pH 7.4. High potassium Krebs solution with the concentration of K<sup>+</sup> ions (80 mM) was prepared by replacing the required amount of Na<sup>+</sup> ions in a standard Krebs solution with the equimolar amount of K<sup>+</sup> ions. Acetylcholine (AC) was used in concentration of 10<sup>-5</sup> M (Sigma).

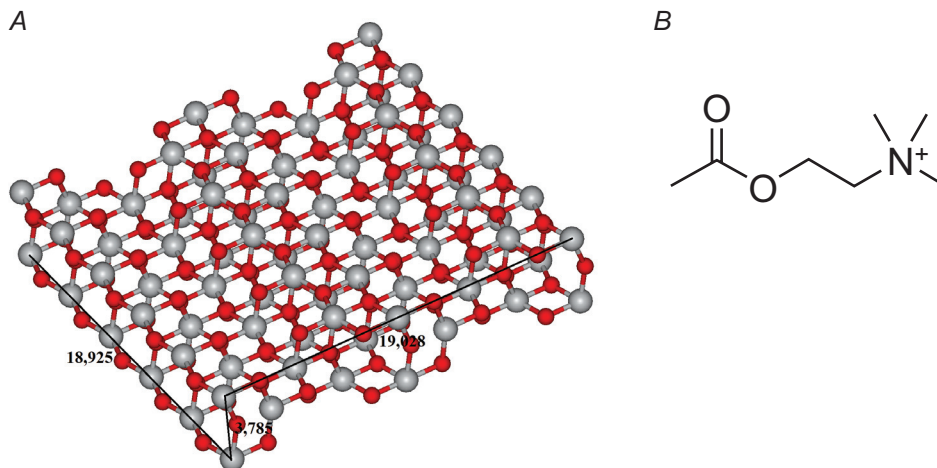
TiO<sub>2</sub> nanoparticles (PlasmaChem GmbH, D-12489 Berlin, Germany) were used in the form of nanopowder (a mixture of rutile and anatase), the size of particles – (4–8) nm and (1–3) nm, a specific surface – (50 ± 10) m<sup>2</sup>/g; purity > 99.5 %, content of Al<sub>2</sub>O<sub>3</sub> < 0.3 wt; SiO<sub>2</sub> < 0.2 wt. The nanopowder of TiO<sub>2</sub> was previously resuspended in dimethylsulfoxide (DMSO) assuming the presence of 0.25 % DMSO in a final volume. Likewise all control solutions contained 0.25% DMSO. A destruction of the aggregates of TiO<sub>2</sub> nanoparticles in the suspension was performed using the ultrasound processing at 37 kHz frequency. The physiological solutions used in the experimental tencometric equipment were flowing ones, and thermostated at 37 °C.

The mechanokinetic analysis of a spontaneous contractive activity of smooth muscle stripes was conducted via calculations using the frequencies of preparation contractions for 10 min; the averaged value of the contraction-relaxation cycle; the duration of some contraction fragments: a contraction phase, a relaxation phase; an asymmetry coefficient; MU index of contractions; AU index of contractions. The method [4] was also used to carry out the kinetic analysis of spontaneous contractions-relaxations of muscle preparations induced by high potassium Krebs solution and acetylcholine with the estimation of normalized maximal velocities of contractions (V<sub>nc</sub>)-relaxations (V<sub>nr</sub>).

A statistical analysis of the experiment results was performed using OriginPro 8 program. The unpaired version of Student's *t*-test was used to determine the reliable

differences between the mean values of two samplings. Multiple comparisons were performed using the parametric one-factor dispersion analysis. The results were considered reliable on condition of the probability value of  $P$  under 5 % ( $P < 0.05$ ). The results were presented in the paper as the arithmetic mean  $\pm$  standard error of the mean value,  $n$  – number of experiments.

For conducting the molecular docking of nanosized  $\text{TiO}_2$  to the extracellular parts of muscarinic M2 and M3 types cholinoreceptors involved a use of the spatial structure of  $\text{TiO}_2$  nanoparticle in the form of anatase [12] which according to literature data [27] was constructed using Discovery Studio Visualizer program, versions 2.0 and 2.5 (Accelrys Software Inc. – <http://accelrys.com/>). Using data about the crystallography of anatase, the following parameters of the elementary cluster of  $\text{TiO}_2$  were used in our work:  $A = B = 3.785 \text{ \AA}$ ;  $C = 9.514 \text{ \AA}$ , where  $A, B, C$  – the lengths of the crystalline grid.  $a = b = \gamma = 90^\circ$ , where  $a, b, \gamma$  – dimensions of crystal angles. A spatial symmetry group (the combination of symmetry transformations, remarkable for the atomic structure of crystals) of  $\text{TiO}_2$   $I4(1)/amd$  [9, 28]. The dimensions of the surface of  $\text{TiO}_2$  nanoparticle obtained after the simulation of the spatial structure were as follows:  $(18.925 \times 3.785 \times 19.028) \text{ \AA}^3$  (Fig. 1A).

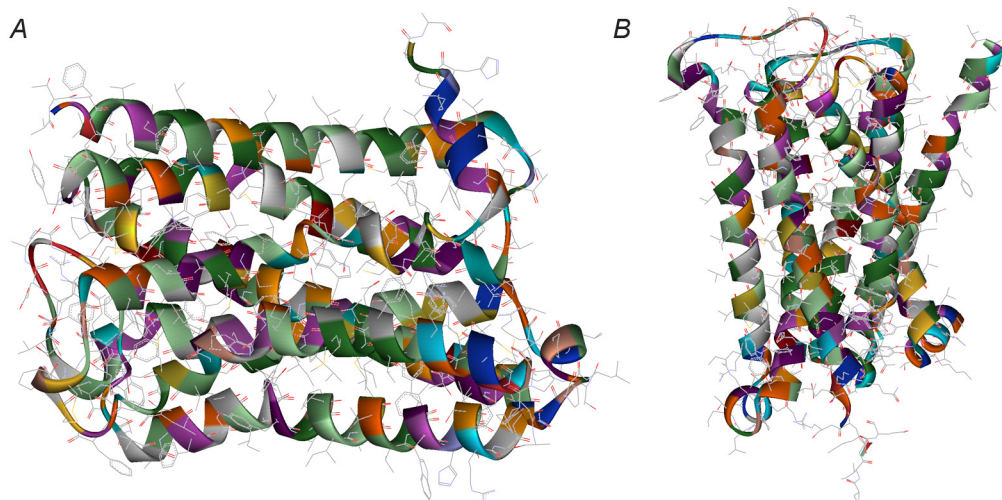


**Fig. 1.** A spatial structure of the nanoparticle of  $\text{TiO}_2$  anatase  $(18.925 \times 3.785 \times 19.028) \text{ \AA}^3$  (A) and structural formula of acetylcholine ( $\text{C}_7\text{H}_{16}\text{NO}_2$ ) (B)

**Рис. 1.** Просторова структура наночастинки анатазу  $\text{TiO}_2$   $(18.925 \times 3.785 \times 19.028) \text{ \AA}^3$  (A) і структурна формула ацетилхоліну ( $\text{C}_7\text{H}_{16}\text{NO}_2$ ) (B)

The information about spatial structure of the acetylcholine molecule (Fig. 1B) (PubChem CID: 187. Molecular formula:  $\text{C}_7\text{H}_{16}\text{NO}_2$ . Molecular weight: 146.21 g/mol.) was received from the PubChem database of chemical compounds and mixtures [16, 36].

The spatial structure of muscarinic acetylcholine M2 receptor (Fig. 2A) was taken from the Protein Data Bank database (PDB) (accessed at <https://www.rcsb.org/pdb/home/home.do>) that is kept in the record 4MQS. This record is not complete; amino acid residues got into the investigated crystal from 19 to 215 and from 377 to 456. 4DAJ is the record number of the M3 type muscarinic acetylcholine receptor structure (Fig. 2B) in the PDB database. The investigated crystal includes from 64 to 259; from 482 to 485, and 489 to 556 amino acid residues.



**Fig. 2.** Spatial structures of the muscarinic acetylcholine M2 type receptor (taken from the record 4MQS of the Protein Data Bank database) (A) and muscarinic acetylcholine M3 type receptor (taken from the record 4DAJ of the Protein Data Bank database) (B)

**Рис. 2.** Просторові структури мускаринового ацетилхолінового рецептора М2 типу (взято з запису 4MQS бази даних Protein Data Bank) (A) і мускаринового ацетилхолінового рецептора М3 типу (взято з запису 4DAJ бази даних Protein Data Bank) (B)

The search for and analysis of interaction sites for muscarinic acetylcholine M2 and M3 receptors to the titanium dioxide nanoparticle and to the acetylcholine was conducted using PatchDock, the algorithm of molecular docking based on geometry [7]. This algorithm consists of three main stages: Molecular Shape Representation, Surface Patch Matching and Filtering and Scoring. Taking two molecules into consideration, PatchDock calculates three-dimensional transformations of one molecule regarding another one with the purpose of complementing the form of the surface, minimizing a number of steric collisions. The service is available at <http://bioinfo3d.cs.tau.ac.il/PatchDock/>. The visualization and analysis of the contact surfaces were performed using Discovery Studio Visualizer software, versions 2.0 and 2.5.

## RESULTS AND DISCUSSION

The mechanisms of the pace-maker activity of interstitial cells of Cajal play a relevant role in regulating the motility of gastrointestinal tract along with the inhibiting and exciting non-cholinergic and exciting cholinergic neurons of intramural nervous system. The functioning of these cells is based on a process of releasing calcium ions into the cytoplasm from the sarcoplasmic reticulum and their absorption by the mitochondria; the frequency of concentration fluctuations of the ions being of the same order with pace-maker currents, and hence, with the amplitude-frequency characteristics of spontaneous contractions of smooth gastrointestinal muscles [10, 11, 30, 37]. Taking the abovementioned into consideration, the tenzometric method was used in our work to study a spontaneous contractive activity of isolated smooth muscle stripes (SMS) of circular smooth muscles of rats antrum in the control and at the cumulative effect of TiO<sub>2</sub> suspension (10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> mg/ml) with the size of nanoparticles of (4–8) nm and (1–3) nm as stated above [17, 23, 32], are structures with excessive energy and high



chemical activity. During 60 min of registration, the level of the basal tone of spontaneous contractions of muscle preparations remained stable. Every 10 min of registration, the amplitude of spontaneous contractions of muscle preparations changed in the range from 2 mN to 16.5 mN,  $n = 10$ ,  $p < 0.05$ . A distribution by frequencies with two significant maximums took place within the indicated interval of amplitudes:  $(21.4 \pm 1.6) \%$  with the amplitude of 4 mN and  $(14.3 \pm 0.9) \%$  with the amplitude of 11.5 mN. It is evident that the contraction of muscle preparations with low amplitude was observed more frequently, compared to the contractions with high amplitude, which is in perfect agreement with the literature data [30, 31]. A average value of the amplitude of spontaneous contractions was  $(10.3 \pm 0.8)$  mN. The frequency of muscle preparation contractions calculated in the control for 10 min was  $(12 \pm 0.58)$ ; the averaged value for the duration of the contraction-relaxation cycle (contraction act) was  $(26.5 \pm 1.86)$  s, the duration of some contraction fragments was as follows: contraction phase –  $(12.25 \pm 0.7)$  s., relaxation phase –  $(14.3 \pm 0.6)$  s.; asymmetry coefficient –  $(1.12 \pm 0.06)$ ; MU index of contractions –  $(123.6 \pm 10.3)$ ; AU index of contractions –  $(3275.4 \pm 209.9)$ .

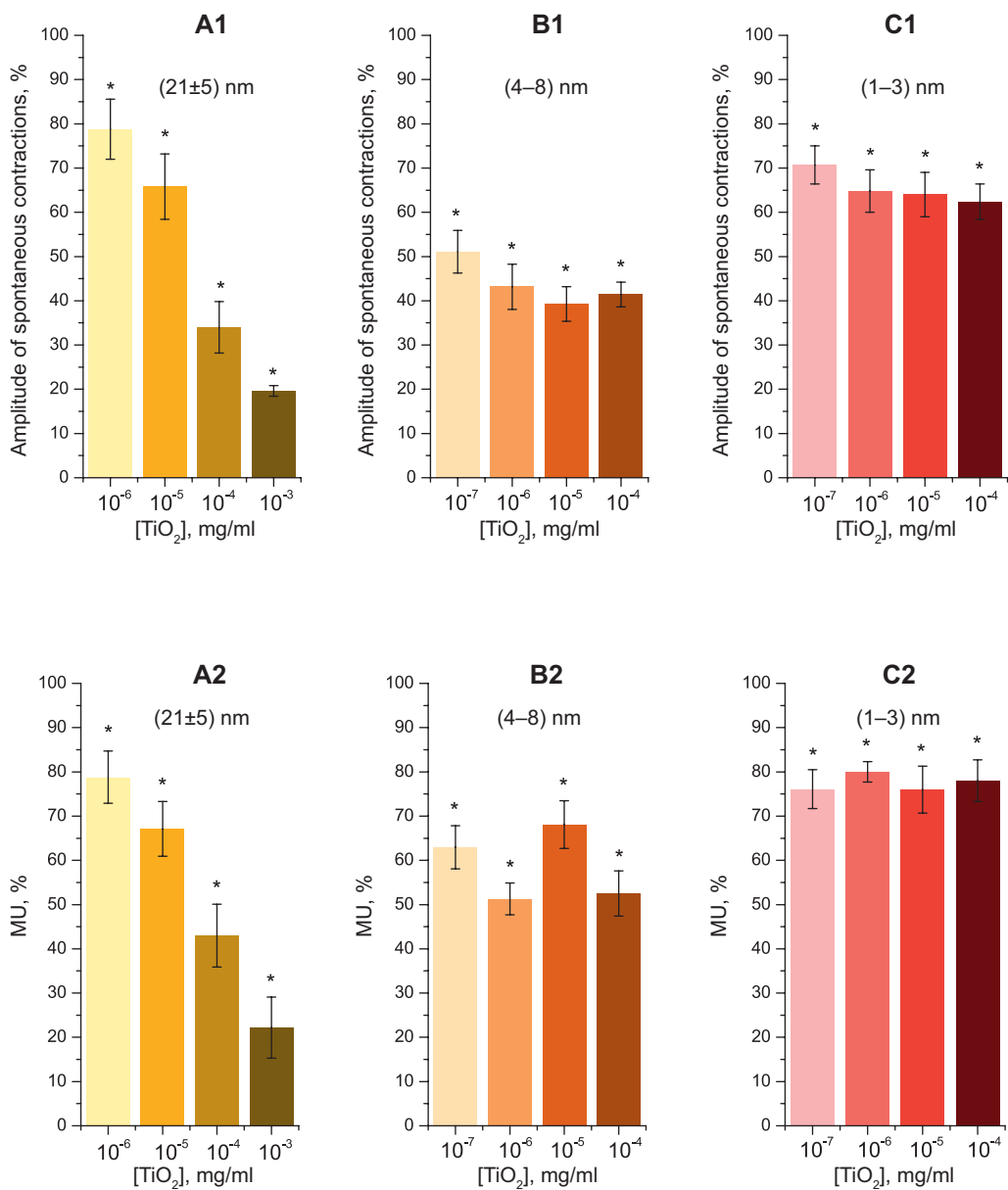
The next experiment was aimed at investigating changes in the abovementioned parameters of kinetic analysis for spontaneous contractive activity of smooth muscles at the cumulative effect of titanium dioxide suspension with the size of particles of (4–8) nm. The time of  $\text{TiO}_2$  application in each concentration was 30 min. It was established (Fig. 3B1) that the presence of  $\text{TiO}_2$  in normal Krebs solution in the concentrations of  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$  mg/ml compared to the control accepted as 100 %, leads to a decrease in averaged value of the amplitude of spontaneous contractions, the value of which was, respectively,  $(51.0 \pm 4.8) \%$ ;  $(43.2 \pm 5.1) \%$ ;  $(39.3 \pm 3.9) \%$ ;  $(41.4 \pm 2.8) \%$ ,  $n = 10$ ,  $p < 0.05$ . The analysis of a frequency of muscle preparation contractions during the interval of 10 min effect of  $\text{TiO}_2$  in the abovementioned concentrations demonstrated that, compared to control, there is an increase in the value of this parameter, the highest one being at a  $\text{TiO}_2$  concentration of  $10^{-5}$  mg/ml namely  $(129.2 \pm 8.8) \%$ ,  $n = 10$ ,  $p < 0.05$ . The analysis of the duration of separate fragments of spontaneous contractions-relaxations and kinetic regularities of their course at the effect of  $\text{TiO}_2$  compared to the control demonstrated that a reliable reduction in duration of, for instance, the contraction phase, compared to the control, occurs when this nanosized material is applied in the concentrations of  $10^{-6}$  and  $10^{-5}$  mg/ml. Compared to the control, the value of this parameter was  $(64.4 \pm 3.9) \%$ ,  $(74.1 \pm 5.9) \%$ ,  $n = 10$ ,  $p < 0.05$ , respectively. At the effect of  $\text{TiO}_2$  in the concentrations of  $10^{-7}$  and  $10^{-4}$  there were no statistically significant changes compared to the control of contraction phase duration  $((92.4 \pm 7.3) \%$  and  $(91.1 \pm 9.3) \%$ ,  $n = 10$ ,  $p < 0.05$ , respectively). A duration of relaxation phase for spontaneous contractions of muscle preparations decreases compared to the control at the effect of this nanosized material in the concentrations of  $10^{-6}$  and  $10^{-5}$  mg/ml:  $(77.6 \pm 7.4) \%$ , and  $(77.2 \pm 6.9) \%$ ,  $n = 10$ ,  $p < 0.05$ , respectively. The calculation of the MU and AU indices of contractions demonstrated (Fig. 3B2, 3B3) that their value decreases at the effect of  $\text{TiO}_2$  compared to the control in all the investigated concentrations: compared to the control, MU index acquired the following values:  $(62.9 \pm 4.9) \%$ ;  $(60.1 \pm 8.1) \%$ ;  $(64.6 \pm 8.7) \%$  and  $(52.5 \pm 5.1) \%$ ,  $n = 10$ ,  $p < 0.05$ , and at the same concentrations AU index was  $(58.5 \pm 4.4) \%$ ;  $(46.3 \pm 4.3) \%$ ;  $(48.3 \pm 3.8) \%$ ; and  $(46.5 \pm 2.8) \%$ ,  $n = 10$ ,  $p < 0.05$ . The data, presented with the consideration of scientific literature [4] regarding the content of MU and AU indices, indicate that the total efficiency of the contractive activity of muscle

preparations in the presence of TiO<sub>2</sub> nanoparticle suspension in the mentioned concentrations, decreases compared to the control. In all the conditions the level of the muscle tone remained unchanged.

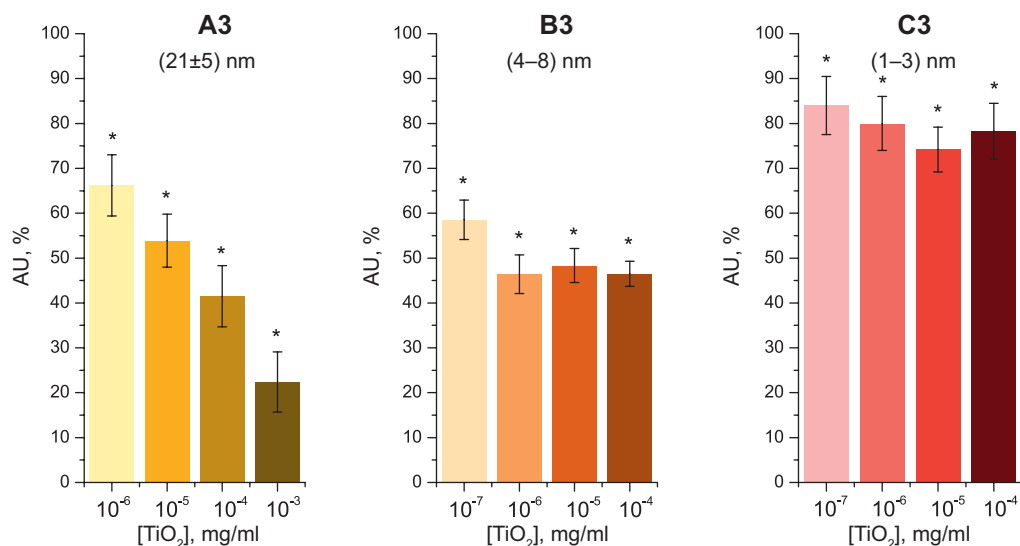
In the next experiment, the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (1–3) nm on spontaneous contractive activity of circular stomach smooth muscles of rats' antrum was studied. Fig. 3C1, 3C2 and 3C3 present the results of estimating mechanokinetic parameters, registered in the experiments on spontaneous contractive activity of stomach smooth muscles at the effect of TiO<sub>2</sub> suspension with the abovementioned size of nanoparticles in the concentrations of 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup> mg/ml. It was established that in these conditions, similar to the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (4–8) nm, the average value of the amplitude of smooth muscle stripes spontaneous contractions is accompanied with the decrease in its value, compared to the control: (70 ± 4.3) %; (64.8 ± 4.8) %; (64.5 ± 5.3) % and (62.4 ± 4) %, respectively, n = 10, p < 0.05. The frequency of preparation contractions for 10 min in these conditions was somewhat higher compared to the control and, when used with the concentrations of TiO<sub>2</sub> for the experiment, was (118.0 ± 8.9) %; (110.0 ± 10.3) %; (126.7 ± 9.8) %; and (125.0 ± 7.7) %, n = 10, p < 0.05. There were no statistically significant changes in the duration of phases of spontaneous contractions and phases of relaxation of smooth muscle stripes. As seen in Fig. 3C2 and 3C3, in these conditions the MU and AU indices of contractions for such muscle preparations were below the control (for MU in the presence of TiO<sub>2</sub> in the concentrations of 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup> mg/ml these parameters were respectively: (76.1 ± 4.4) %; (80.0 ± 2.3) %; (76.1 ± 5.3) % and (78.0 ± 4.7) %, n = 10, p < 0.05; for AU in the presence of TiO<sub>2</sub> in the concentrations of 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> та 10<sup>-4</sup> mg/ml they were: (84.0 ± 6.5) %; (80.1 ± 5.9) %; (74.2 ± 5.0) % and (78.3 ± 6.2) %, n = 10, p < 0.05 regarding control), which indicates their loss of efficiency. Changes in the kinetic parameters of spontaneous contractive activity of smooth muscles at the effect of TiO<sub>2</sub> suspension with the size of particles of (4–8) nm and (1–3) nm, demonstrated in the experiments, are likely to be related to their modulating effect on the pace-maker activity of interstitial cells, the mechanism of which, according to the literature data [26, 37], is related to a release of calcium ions from the sarcoplasmic reticulum and their being absorption by the mitochondria.

The following parameters were selected for a comparative analysis of the changes in the kinetics of spontaneous contractive activity of circular stomach smooth muscle of rats antrum at the effect of TiO<sub>2</sub> suspension with the particle sizes of (21 ± 5) nm [33]; (4–8) nm and (1–3) nm – the average value of the spontaneous contractions amplitude and MU and AU indices of contractions. As seen in Fig. 3, in presence of TiO<sub>2</sub> suspension with nanoparticles of (21±5) nm, (4–8) nm, (1–3) nm, there are one-direction changes in the average value of the amplitude of smooth muscle stripes spontaneous contractions, MU and AU indices of contractions, related to a reduction in their value compared to the control. The least changes in the mentioned parameters of spontaneous contractions of smooth muscles were registered at the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (1–3) nm. In addition, if these changes in amplitude of spontaneous contractions are of dose-dependent nature at the effect of TiO<sub>2</sub> suspensions in different concentrations with the size of nanoparticles of (21 ± 5) nm, in case of applying suspensions of this nano-material of (4–8) and (1–3) nm, the effect is almost absent.

The next series of experiments was aimed at investigating changes in high potassium contraction of circular stomach smooth muscles of rats' antrum at the cumulative



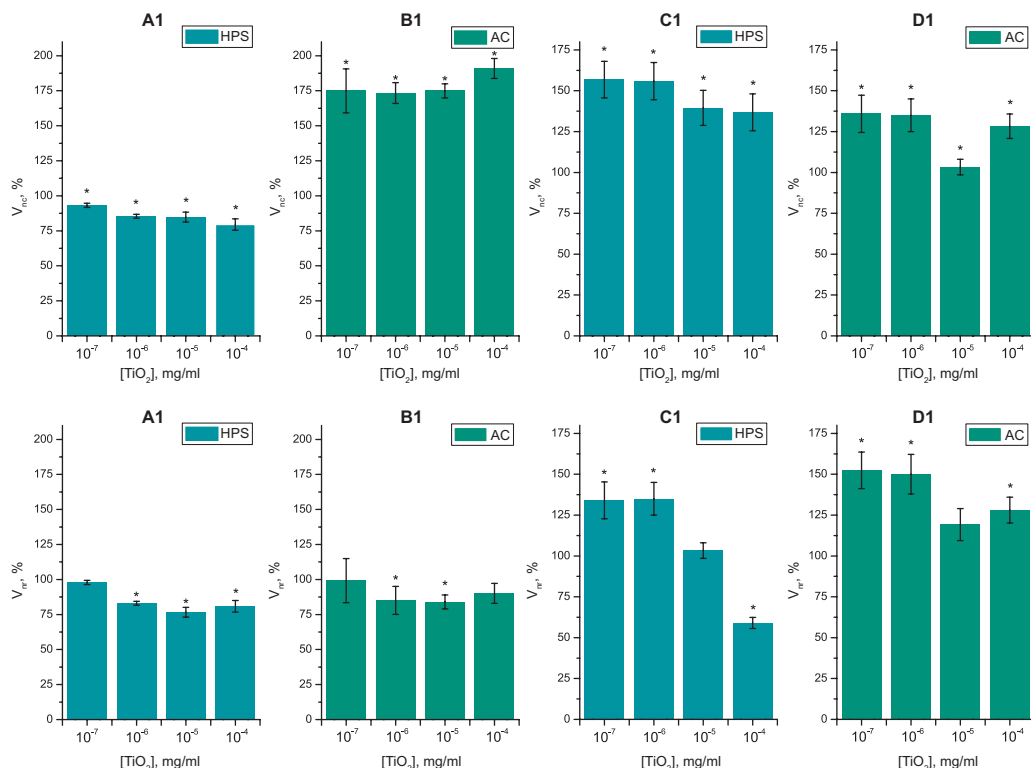




**Fig. 3.** The histograms of changes in the kinetic analysis parameters of a spontaneous contractive activity (the average value of amplitude of the spontaneous contractions; MU and AU indices of circular stomach smooth muscles of rats antrum at the effect of TiO<sub>2</sub> suspension (TiO<sub>2</sub>) with the following size of nanoparticles: (21 ± 5) nm (A1, A2, A3); (4–8) nm (B1, B2, B3) and (1–3) nm (C1, C2, C3). A value of kinetic analysis parameters in the control was accepted as 100 %. \* – p < 0.05

**Рис. 3.** Гістограми змін параметрів кінетичного аналізу спонтанної скорочувальної активності (середнє значення амплітуди спонтанних скорочень; показники скоротливого індексу в одиницях Монтевідео (MU) та в Олександрійських одиницях (AU)) кільцевих гладеньких м'язів антрального відділу шлунка щурів за дії суспензії діоксиду титану (TiO<sub>2</sub>) з розміром наночастинок відповідно: (21 ± 5) нм (A1, A2, A3); (4–8) нм (B1, B2, B3) та (1–3) нм (C1, C2, C3). За 100 % прийнято значення параметрів кінетичного аналізу в контролі. \* – p < 0,05

effect of TiO<sub>2</sub> suspension with the size of the nanoparticles of (4–8) nm. The time of TiO<sub>2</sub> suspension application in each concentration was 30 min. In response to the application of the high potassium (80 mM) Krebs solution, muscle preparations of control groups of rats developed contractions-relaxations, the averaged value of phase component of which was (19.6 ± 1.3) mN, whereas the value for a tonic component was (18.5 ± 0.6) mN, n = 10. A ratio of a phase component and ta tonic one was (1.02 ± 0.08). The kinetic analysis of K<sup>+</sup>-induced contractions of muscle preparations in the control demonstrated that the normalized maximal velocity of the contraction phase was (5.69 ± 0.47) min<sup>-1</sup>, whereas the normalized maximal velocity of the relaxation phase was (1.33 ± 0.11) min<sup>-1</sup>. The abovementioned parameters of high potassium contractions in the control remained stable during the whole period of registering contractions. Our experiments established that at the effect of TiO<sub>2</sub> suspension in the concentrations of 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup> mg/ml, there were no significant changes in the phase component of high potassium contractions compared to the control ((113.3 ± 8.1) %; (101.5 ± 6.9) %; (86.7 ± 7.2) %; (83.2 ± 5.5) %). There were no changes in a ratio of the phase component of these contractions and the tonic component. Fig. 4A1, 4A2 demonstrate the histograms of changes in such parameters of the kinetic analysis of high potassium contractions, as V<sub>nc</sub> and V<sub>nr</sub> at the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (4–8) nm.



**Fig. 4.** The histograms of changes in the kinetic analysis parameters for the normalized maximal velocity ( $V_{nc}$ ) of contractions of circular stomach smooth muscles (SM) induced by high potassium (80 mM) Krebs solution at the cumulative effect of  $TiO_2$  suspension with the sizes of nanoparticles of (4–8) nm (A1) and (1–3) nm (C1), respectively; A2, C2 – normalized maximal velocities of relaxation ( $V_{nr}$ ). B1 and D1 – histograms of changes in  $V_{nc}$  of SM contraction induced by the acetylcholine ( $10^{-5}$  M) at the cumulative effect of  $TiO_2$  suspension with the abovementioned sizes of nanoparticles; B2, D2 – normalized maximal velocities of relaxation. The value of kinetic analysis parameters in the control was accepted as 100%. \* –  $p < 0.05$ . The Figure presents the concentrations of  $TiO_2$  suspension in mg/ml. Time of  $TiO_2$  application in each concentration was 30 min

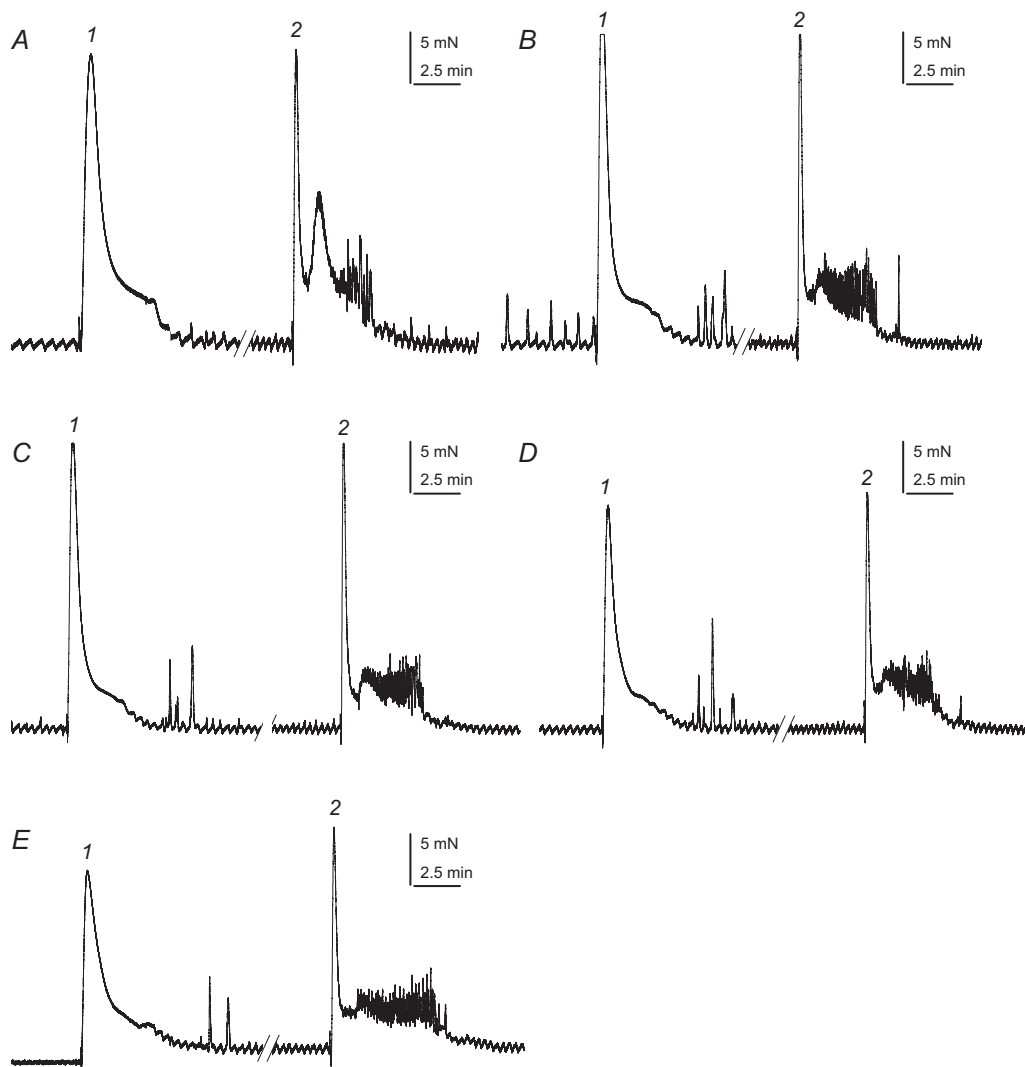
**Рис. 4.** Гістограми змін параметрів кінетичного аналізу нормованої максимальної швидкості ( $V_{nc}$ ), спричиненого гіперкалієвим (80 мМ) розчином Кребса скорочення кільцевих гладеньких м'язів (ГМ) шлунка за кумулятивної дії суспензії діоксиду титану ( $TiO_2$ ) з розмірами наночастинок: (4–8) нм (A1) та (1–3) нм (C1), відповідно; A2, C2 – нормовані максимальні швидкості розслаблення ( $V_{nr}$ ). B1 та D1 – гістограми змін  $V_{nc}$ , зумовленого ацетилхоліном ( $10^{-5}$  М) скорочення ГМ за кумулятивної дії суспензії  $TiO_2$  з зазначеними вище розмірами наночастинок; B2, D2 – нормовані максимальні швидкості розслаблення. За 100% прийнято значення параметрів кінетичного аналізу у контролі. \* –  $p < 0,05$ . На рисунку наведено концентрації суспензії  $TiO_2$  у мг/мл. Час аплікації  $TiO_2$  у кожній з концентрацій становив 30 хв

It was established that the abovementioned concentrations of  $TiO_2$  cause a reduction in normalized maximal velocity of the contraction, compared to the control, with the corresponding values of  $(93.2 \pm 1.5) \%$ ;  $(85.4 \pm 1.4) \%$ ;  $(84.8 \pm 3.5) \%$ ;  $(79.5 \pm 4.1) \%$ ,  $n = 10$ ,  $p < 0.05$ . A kinetic analysis of  $K^+$ -induced contractions of muscle preparations demonstrated that there is a simultaneous reduction in the value of their normalized maximal velocity of relaxation at the effect of  $TiO_2$  in the following concentrations:  $10^{-6}$ ,

10<sup>-5</sup> and 10<sup>-4</sup> mg/ml: (83 ± 1.4) %; (76.6 ± 3,3) %; (80.9 ± 4,2) %; n = 10, p < 0.05, whereas the effect of concentration of this nanosized material of 10<sup>-7</sup> mg/ml did not lead to any statistically reliable changes in this parameter (97.9±1.7) %. It is known [26] that high potassium in Krebs solution causes depolarization of the membrane, the influx of extracellular calcium ions into smooth muscle cells, as well as a release of these cations from ryanodine-sensitive stores of sarcoplasmic reticulum, the activation of the contraction apparatus, that leads to a development of their contraction, whose velocity decreased at the effect of TiO<sub>2</sub> compared to the control. At the same time, the deviation of the intracellular concentration of calcium ions from their basal level during membrane depolarization induced by high potassium Krebs solutions, at the effect of TiO<sub>2</sub> suspension remained at the level of the control (at the effect of TiO<sub>2</sub> the amplitude of high potassium contractions of SMS was at the basal level). Compared to the control, the inhibition of the normalized maximal velocity of smooth muscle contraction induced by high potassium Krebs solution, also occurred at the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (21 ± 5) nm (Fig. 6). The calculations of a ratio of the normalized maximal velocities of contractions and relaxations at the effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (4–8) nm in the abovementioned concentrations demonstrated that its value was in a range of the control. When TiO<sub>2</sub> suspension with the size of nanoparticles of (21 ± 5) nm was applied in the concentration of 10<sup>-4</sup> mg/ml, there was more than 1.5-fold decrease (p < 0.05) in the value of this parameter compared to the control. In case of TiO<sub>2</sub> concentrations of 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> mg/ml this parameter remained without any changes.

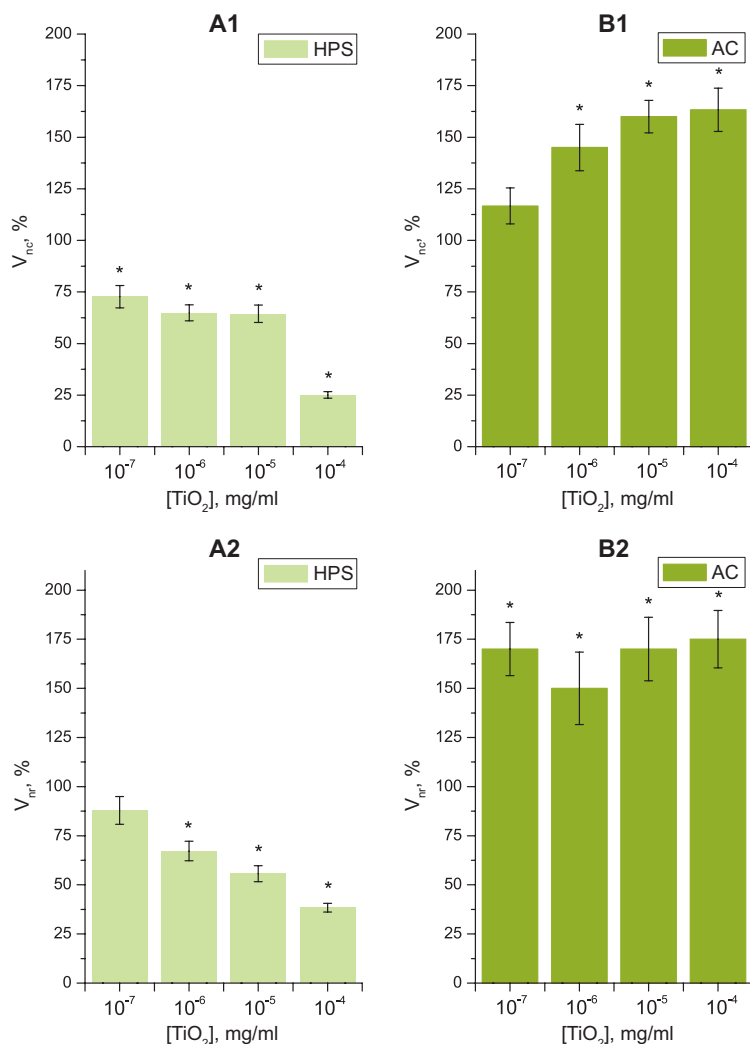
Fig. 4 and Fig. 5 present the histograms and contractions of stomach smooth muscles of rats antrum registered by the tensometric method that were induced by high potassium (80 mM) Krebs solution at the cumulative effect of TiO<sub>2</sub> suspension with the size of nanoparticles of (1–3) nm. Similarly to previous experiments, time interval of applying this nanosized material in each concentration was 30 min. A kinetic analysis of high potassium contraction phase demonstrated that in these conditions the concentrations of TiO<sub>2</sub> of 10<sup>-7</sup>; 10<sup>-6</sup>; 10<sup>-5</sup> and 10<sup>-4</sup> mg/ml led to an increase in the velocity of contraction response intensification for GM compared to the control, amounting to (156.8 ± 11.2) %; (155.9 ± 11.4) %; (139.5 ± 10.7) %; (136.8 ± 11.3) %, n = 10, p < 0.05. At the same time, a kinetic analysis of the relaxation phase demonstrated that at the concentration of this nanosized material of 10<sup>-7</sup> and 10<sup>-6</sup> mg/ml there was also an increase in its maximal velocity compared to the control ((134 ± 11.3) % and (133.9 ± 8.4) %, n = 10, p < 0.05). Whereas in case of the concentration of 10<sup>-5</sup> mg/ml there was no statistically significant changes in the value of this parameter (98.2 ± 4.6) %, and at 10<sup>-4</sup> mg/ml this parameter amounted to (59.1 ± 3.4) %, n = 10, p < 0.05. As for the changes in the amplitude of K<sup>+</sup>-induced contractions in these conditions, there was an increase in its value at the effect of this nanosized material in the concentrations of 10<sup>-7</sup> and 10<sup>-6</sup> mg/ml compared to the control, amounting to the following values: (144.9 ± 9.3) % and (138.9 ± 7.8) %, n = 10, p < 0.05. At the effect of this nanosized material in concentration of 10<sup>-5</sup> mg/ml there were no statistically reliable changes in this parameter ((99.4±8.3) %), and there was a decrease in it at the concentration of 10<sup>-4</sup> mg/ml ((73.7 ± 5.7) %, n = 10, p < 0.05). At the effect of TiO<sub>2</sub> suspension (10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> mg/ml) with the size of nanoparticles of (1–3) nm, there was an impairment of ratio of the velocities of contraction-relaxation of SMS induced by high potassium Krebs solution that was accompanied by its increase, and at TiO<sub>2</sub> concentration of 10<sup>-4</sup> mg/ml, this parameter exceeded the control more than two-fold (Fig. 7). As

stated above, at the effect of  $\text{TiO}_2$  suspension with the size of nanoparticles of (4–8) nm and  $(21 \pm 5)$  nm (concentrations of  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  mg/ml), this ratio remained in a range of the control, whereas there was a decrease in this parameter in  $\text{TiO}_2$  concentration of  $10^{-4}$  mg/ml with the size of  $(21 \pm 5)$  nm compared to the control  $(64.9 \pm 5.4)\%$ ,  $n = 10$ ,  $p < 0.05$ .



**Fig. 5.** The contractions of circular stomach smooth muscles of rats antrum, induced by high potassium Krebs solution (80 mM) (1) and acetylcholine ( $10^{-5}$  M) (2) in the control (A) and at the cumulative effect (B, C, D, E) of  $\text{TiO}_2$  suspension ( $\text{TiO}_2$ ) with the size of nanoparticles of (1–3) nm in the following concentrations:  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$  mg/ml. Time of  $\text{TiO}_2$  application in each concentration was 30 min

**Рис. 5.** Викликані гіперкалієвим (80 мМ) розчином Кребса (1) та ацетилхоліном ( $10^{-5}$  М) (2) скорочення кільцевих гладеньких м'язів антрального відділу шлунка щурів у контролі (A) та за кумулятивної дії (B, C, D, E) суспензії діоксиду титану ( $\text{TiO}_2$ ) з розміром наночастинок (1–3) нм у концентраціях відповідно:  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  та  $10^{-4}$  мг/мл. Час аплікації  $\text{TiO}_2$  у кожній з концентрацій становив 30 хв



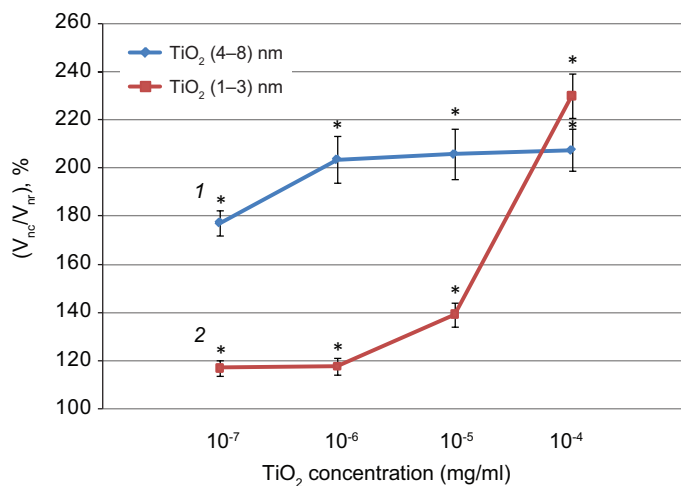
**Fig. 6.** The histograms of changes in the kinetic analysis parameters for the normalized maximal velocity ( $V_{nc}$ ) of the contractions of circular stomach smooth muscles (SM) induced by high potassium (80 mM) Krebs solution at the cumulative effect of TiO<sub>2</sub> suspension with the sizes of nanoparticles of (21 ± 5) nm (A1); A2 – normalized maximal velocity of relaxation ( $V_{nr}$ ). B1 – the histograms of changes in  $V_{nc}$  of SM contraction induced by acetylcholine (10<sup>-5</sup> M) at the cumulative effect of TiO<sub>2</sub> suspension with the abovementioned sizes of nanoparticles; B2 – normalized maximal velocities of relaxation. A value of the kinetic analysis parameters in the control was accepted as 100 %. \* –  $p < 0.05$ . The Figure presents the concentrations of TiO<sub>2</sub> suspension in mg/ml. Time of TiO<sub>2</sub> application in each concentration was 30 min

**Рис. 6.** Гістограми змін параметрів кінетичного аналізу нормованої максимальної швидкості ( $V_{nc}$ ), спричиненого гіперкалієвим (80 мМ) розчином Кребса скорочення кільцевих гладеньких м'язів (ГМ) шлунка за кумулятивної дії суспензії діоксиду титану (TiO<sub>2</sub>) з розміром наночастинок (21 ± 5) нм (A1); A2 – нормована максимальна швидкість розслаблення ( $V_{nr}$ ). B1 – гістограми змін  $V_{nc}$ , зумовленого ацетилхоліном (10<sup>-5</sup> М) скорочення ГМ за кумулятивної дії суспензії TiO<sub>2</sub> з зазначеним вище розміром наночастинок; B2 – нормовані максимальні швидкості розслаблення. За 100 % прийнято значення параметрів кінетичного аналізу в контролі. \* –  $p < 0,05$ . На рисунку наведено концентрації суспензії TiO<sub>2</sub> у мг/мл. Час аплікації TiO<sub>2</sub> у кожній з концентрацій становив 30 хв



It is known [3, 8] that the parasympathetic control of the contractive activity of smooth muscles occurs with an involvement of the acetylcholine, the excitation neuro-mediator. Taking the abovementioned into consideration, the contraction of preparations of circular smooth muscles of antrum activated by acetylcholine, the agonist of muscarinic cholinoreceptors in the concentration of  $10^{-5}$  M was registered in the work. It was established (Fig. 4) that in the control an averaged value of the phase component of acetylcholine-induced contraction of SMS was  $(14 \pm 1.2)$  mN and its ratio to the tonic component –  $(1.25 \pm 0.05)$ ,  $n = 10$ . Here the estimated normalized maximal velocity of the contraction phase was  $(7.65 \pm 0.6)$   $\text{min}^{-1}$ ,  $n = 10$  whereas the normalized maximal velocity of the relaxation phase was  $(2.13 \pm 0.2)$   $\text{min}^{-1}$ . In the next experiment, the cumulative effect of  $\text{TiO}_2$  suspension with the size of nanoparticles of (4–8) nm was studied in concentrations of  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$  mg/ml. Time of  $\text{TiO}_2$  application in each concentration was 30 min. It is known [3, 20] that the phase component of contractions, induced by acetylcholine in stomach smooth muscles is formed both due to the increase in the basal level of intracellular concentration of calcium ions that enter smooth muscles of the intestines via the potential-regulated calcium channels of the plasmatic membrane, and due to a release of these cations from the intracellular stores of their storing, as well as due to the involvement of  $\text{Ca}^{2+}$ -independent processes of the phosphorylation of the myosin light chains. In our experiments, phase component of the AC-induced contraction at the effect of  $\text{TiO}_2$  was in the range of control values for this parameter, but the ratio of the phase component of the contraction to its tonic component changed. As stated above, in control, this ratio was  $(1.25 \pm 0.05)$ , whereas in the presence of  $\text{TiO}_2$ , a decrease in the tonic component of the contraction ( $(103.3 \pm 6.1)$  %;  $(83.3 \pm 5.7)$  %;  $(70.5 \pm 3.8)$  %;  $(63.1 \pm 4.4)$  %,  $n = 10$ ) induced by acetylcholine, led to its increase:  $(1.46 \pm 0.04)$ ;  $(1.57 \pm 0.03)$ ;  $(1.65 \pm 0.05)$ ;  $(1.63 \pm 0.03)$ ,  $n = 10$ ,  $p < 0.05$  that, in these conditions, may be caused by the modulation of the mechanisms of decreasing the intracellular concentration of  $\text{Ca}^{2+}$  down to their basal level in smooth muscle cells by this nanosized material. The estimation of the kinetic parameters demonstrated that the normalized maximal velocity of AC-induced contractions of smooth muscles increased more than 1.5-fold compared to the control, the value of which, depending on the concentrations of titanium dioxide, was as follows:  $(174.9 \pm 15.7)$  %;  $(173.4 \pm 14)$  %;  $(174.9 \pm 15)$  %;  $(190.9 \pm 17.1)$  %,  $n = 10$ ,  $p < 0.05$ . One can assume that this increase in the velocity of contractions occurs at the link of transmitting the signal of the agonist acetylcholine: cholinoreceptor – inositol triphosphate ( $\text{IP}_3$ ) – release of  $\text{Ca}^{2+}$  ions from  $\text{IP}_3$  – a sensitive store of the sarcoplasmic reticulum (SR). As for the link of transmitting the signal of the same agonist: cholinoreceptor – membrane depolarization – activation of potential-regulated calcium channels of the plasmatic membrane of smooth muscles of intestines – the release of extracellular calcium ions –  $\text{Ca}^{2+}$ -induced release of  $\text{Ca}^{2+}$  from the rianodine-sensitive store SR [3, 8], thus, as demonstrated above in the studies of the changes of the high potassium contraction at the effect of  $\text{TiO}_2$ , a signal of the agonist is inhibited rather than accelerated in this area. There was an insignificant decrease in the normalized maximal velocity of relaxation for acetylcholine-induced contractions in the experiments:  $(99.2 \pm 15.7)$  %,  $n = 10$ ,  $p < 0.05$ ;  $(85.1 \pm 5.2)$  %;  $(84 \pm 5)$  %;  $(90 \pm 7.1)$  %,  $n = 10$ ,  $p < 0.05$ . Calculations of the ratio of the normalized maximal velocities of contractions and relaxations of smooth muscles induced by acetylcholine at the effect of  $\text{TiO}_2$  suspension with the size of nanoparticles of (4–8) nm in the abovementioned

concentrations, demonstrated that its value increased in the range of the investigated concentrations and exceeded the control almost twice at 10<sup>-4</sup> mg/ml concentration of this material (Fig. 7).



**Fig. 7.** A chart of the dependency of the averaged value of the  $V_{nc}$  to  $V_{nr}$  ratio of the contractions of circular smooth muscles induced by acetylcholine (10<sup>-5</sup> M) (1) and high potassium Krebs solution (80 mM) (2) on the concentration of TiO<sub>2</sub> as a nanosized material: 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup> mg/ml.  $V_{nc}$ ,  $V_{nr}$  – the velocities of contractions and relaxations, respectively

**Рис. 7.** Графік залежності усередненого значення відношення  $V_{nc}$  до  $V_{nr}$  скорочень кільцевих гладеньких м'язів, викликаних ацетилхоліном (10<sup>-5</sup> М) (1) та гіперкалієвим (80 мМ) розчином Кребса (2) від концентрації нанорозмірного матеріалу діоксиду титану: 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> і 10<sup>-4</sup> мг/мл.  $V_{nc}$ ,  $V_{nr}$  – швидкості скорочення та розслаблення, відповідно

The next series of experiments was aimed at investigating the cumulative effect of TiO<sub>2</sub> suspension with the sizes of nanoparticles of (1–3) nm on the contraction of smooth muscle stripes of rats antrum induced by the acetylcholine in the concentration of 10<sup>-5</sup> M. It was established (Fig. 4, 5) that, compared to the control (accepted as 100 %), the effect of TiO<sub>2</sub> in the concentrations of 10<sup>-7</sup> and 10<sup>-6</sup> mg/ml led to an increase in the amplitude of contractions, the value of which was as follows: (132.1 ± 8.4) %, (127.8 ± 7.2) %; n = 10, p < 0.05, whereas in higher concentrations, nanoscale material did not cause statistically significant changes in this parameter: (103.2 ± 7.7) %, n = 10, p < 0.05, at the concentration of 10<sup>-5</sup> mg/ml; and (89.9 ± 8.3) %, n = 10, p < 0.05, at the concentration of 10<sup>-4</sup> mg/ml. Calculations of a value of the normalized maximal velocity of contractions and relaxations of SMS demonstrated that at the TiO<sub>2</sub> concentrations of 10<sup>-7</sup>, 10<sup>-6</sup> mg/ml, an increase in the contraction amplitude occurred not only compared to the control but also to  $V_{nc}$ : (125.9 ± 11.4)% and (135.0 ± 10.0) %, n = 10, p < 0.05 and  $V_{nr}$ : (152.4 ± 11.2) % and (150.0 ± 12.1) %, n = 10, p < 0.05. There were no statistically significant changes in normalized maximal velocity of contractions at the effect of TiO<sub>2</sub> in the concentration of 10<sup>-5</sup> mg/ml compared to the control ((103.3 ± 4.8) %, n = 10, p > 0.05), whereas the velocity of relaxation increased, amounting to (119.2 ± 9.8)%, n = 10, p < 0.05. Increase in  $V_{nc}$  and  $V_{nr}$  of acetylcholine-induced contractions occurred at the concentration of TiO<sub>2</sub> of 10<sup>-4</sup> mg/ml: (128.3 ± 7.9) %, n = 10, p < 0.05 and (128.1 ± 7.9) %, n = 10, p < 0.05, respectively. Calculations of the ratio of the normalized

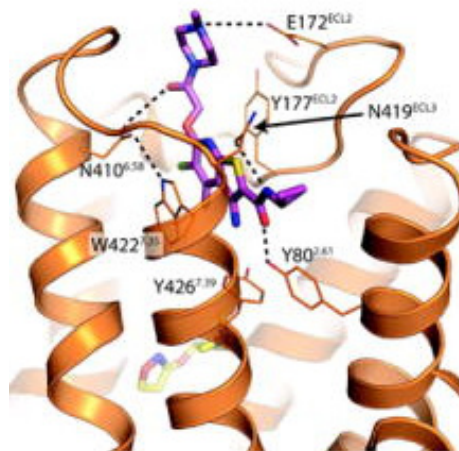
maximal velocities of contractions and relaxations of smooth muscles induced by acetylcholine at the effect of TiO<sub>2</sub> suspension (10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> mg/ml) with the size of nanoparticles of (4–8) nm in the abovementioned concentrations demonstrated that its value was in a control range. This ratio of velocities at the effect of titanium dioxide suspension with the size of nanoparticles of (21 ± 5) nm remained in a control range (Fig. 6). In all the conditions of experiments, the level of muscle tone remained unchanged.

Further on, the molecular docking of the nanosized TiO<sub>2</sub> to the extracellular part of the muscarinic choline M2 type receptor was conducted. According to the results of literature [2, 13], the structure of a non-activated choline M2 type receptor has a large groove located towards the extracellular space that hosts the site to the allosteric modulator. This groove in the receptor structure is located immediately above the orthosteric site of binding the agonist. When the M2 type receptor is activated due to the turn of its TM6 helix, the edges of the groove come close. The movement of TM6 helix ensures the structural bond between three regions of this receptor: the extracellular groove, the orthosteric pocket and the intracellular surface. The structural bond of these three regions demonstrates that the allosteric modulators affect the affinity of the receptor to orthosteric ligands and may directly activate G-proteins as allosteric agonists. It was demonstrated for the muscarinic acetylcholine M2 receptor [19] that its positive allosteric modulator LY2119620 was capable of activating this receptor directly, albeit with lower efficiency compared to, for instance, the orthosteric agonist, the ipexoro. A site of binding ipexoro consists of non-polar (Trp400), polar uncharged (Tyr104, Tyr403, Asn404, Tyr426, Cys429) and polar charged (Asp103) amino acid residues in the ratio of 0.14:0.71:0.14 and the corresponding distribution of Gibbs free energy (kJ/mol): (-0.45); (-0.85); (-0.15). It was calculated via changes in temperature of the helix-coil transition while injecting a specific amino acid into a standard amino acid sequence of the peptide, and was used as a measure of the capability of different amino acids to form a helix [24]. The amino acid composition of binding site for allosteric modulator LY2119620 includes: non-polar Trp422), polar uncharged (Tyr80, Tyr83, Tyr177, Asn410, Asn419, Tyr426), polar charged (Glu172) amino acid residues (Fig. 8) in the following ratios: 0.125:0.75:0.125 and the corresponding distribution of Gibbs free energy (kJ/mol): (-0.45); (-0.9); (-0.27). Taking the abovementioned into consideration, it was interesting to find out the composition of the binding sites for TiO<sub>2</sub> nanoparticle and acetylcholine in structure of the muscarinic choline M2 type receptor with a purpose of determining their functional relevance, to compare them against the already determined and abovementioned binding sites for the orthosteric agonist ipexoro and the allosteric modulator LY2119620 and this receptor.

The amino acid composition and environment of the acetylcholine binding site (Fig. 9) with the highest binding affinity includes the following amino acid residues: Asp103, Tyr104, Ser107, Asn108, Trp155, Ala194, Trp400, Tyr403, Asn404, Cys429, Tyr430. The Geometric shape complementarity score (Score) of this site was 3648, the Approximate interface area (Area) complex was 384.20 Å<sup>2</sup>, the Atomic Contact energy (ACE) [39] was (-132.89). Among amino acids of the site the nonpolar (Trp155, Ala194, Trp400), polar uncharged (Tyr104, Ser107, Asn108, Tyr403, Asn404, Cys429, Tyr430) and polar charged amino acids residues (Asp103) are in the ratio of 0.27:0.64:0.09, and the corresponding distribution of the Gibbs free energy (kJ/mol) is (-1.67); (-1.31); (-0.15).

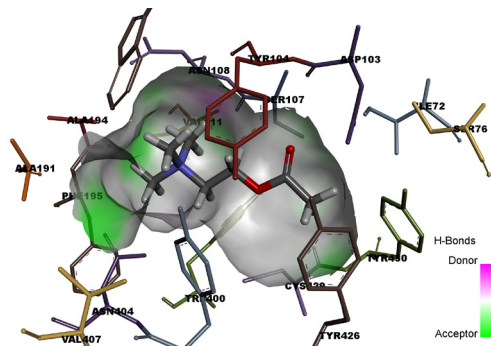
**Fig. 8.** The site of binding the allosteric modulator LY2119620 of the muscarinic acetylcholine M2 type receptor [19]

**Рис. 8.** Сайт зв'язування алостеричного модулятора LY2119620 мускаринового ацетилхолінового рецептора M2 типу [19]



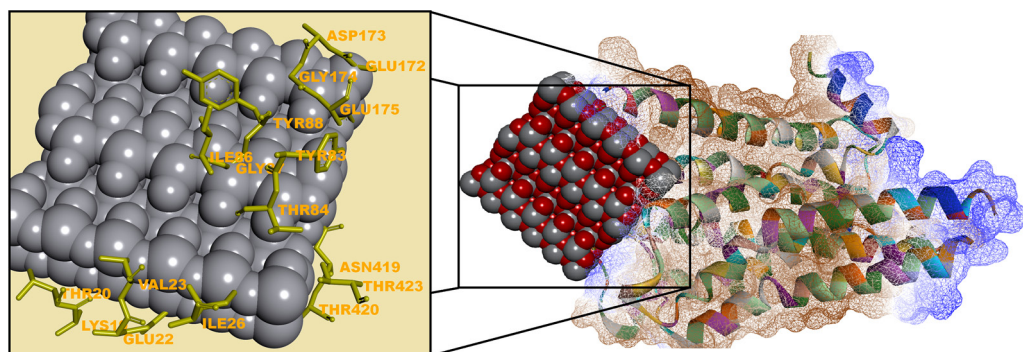
**Fig. 9.** The acetylcholine (PubChem CID: 187) binding to the M2 type muscarinic acetylcholine receptor (Score = 3648, Area = 384.20 Å<sup>2</sup>, ACE = -132.89)

**Рис. 9.** Сайт зв'язування агоніста ацетилхоліну (PubChem CID: 187) з мускариновим ацетилхоліновим рецептором M2 типу (Score = 3648, Area = 384.20 Å<sup>2</sup>, ACE = -132.89)



According to the results of calculations of PatchDock web-server, the amino acid composition and environment of the binding site for TiO<sub>2</sub>, which demonstrated the highest affinity of binding (Fig. 10), included the following amino acid residues: Lys19, Thr20, Glu22, Val23, Ile26, Tyr83, Thr84, Ile86, Gly87, Tyr88, Glu172, Asp173, Gly174, Glu175, Asn419, Thr420, and Thr423. For this site the Geometric shape complementarity score (Score) had a value of 7538, and the Approximate interface area (Area) for complex of the muscarinic acetylcholine M2 type receptor and TiO<sub>2</sub> nanoparticle was 955.40 Å<sup>2</sup>, the Atomic Contact energy (ACE) was (- 53.46).

It was established that a site of binding the TiO<sub>2</sub> nanoparticle to the muscarinic acetylcholine M2 type receptor forms four groups of consecutively located amino acid residues. The first group of amino acids includes Lys19, Thr20, Glu22, Val23, Ile26; the second group of amino acids includes Tyr83, Thr84, Ile86, Gly87, Tyr88; the third group of amino acids includes Glu172, Asp173, Gly174, Glu175; the fourth group of amino acids includes Asn419, Thr420, Thr423. The distances between these groups are 57, 84 and 244 amino acid residues, respectively. Among amino acids of the site are non-polar amino acid residues (Val23, Ile26, Ile86, Gly87, Gly174), polar uncharged (Thr20, Tyr83, Thr84, Tyr88, Asn419, Thr420, Thr423), polar charged (Lys19, Glu22, Glu172, Asp173, Glu175) residues, that are in the ratios of 0.29:0.41:0.29 and the corresponding distribution of Gibbs free energy (kJ/mol) is (-0.73); (-0.6); (-1.61). The amino acid residues with negatively charged radicals prevail among the polar charged amino acids in this site.



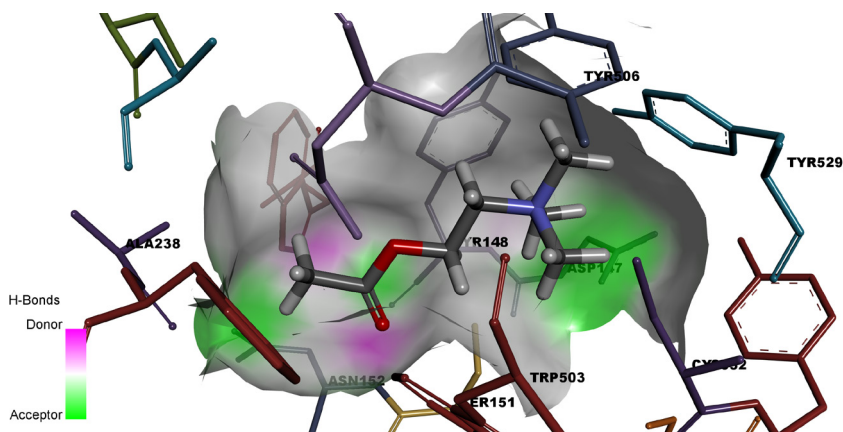
**Fig. 10.** A site of binding the  $\text{TiO}_2$  nanoparticle to the muscarinic acetylcholine M2 type receptor (Score = 7538, Area = 955.40, ACE = - 53.46)

**Рис. 10.** Сайт зв'язування наночастинки  $\text{TiO}_2$  з мускариновим ацетилхоліновим рецептором M2 типу (Score = 7538, Area = 955.40, ACE = - 53.46)

The comparison of the binding site for nanosized  $\text{TiO}_2$  against the binding site for the allosteric modulator LY2119620, the orthosteric agonist ipexoro to the muscarinic choline M2 type receptor demonstrates that their structure is built according to the same principles, namely, a share of the non-polar amino acid residues in each of these sites corresponds to the share of polar charged amino acid residues. In terms of the total Gibbs energy for amino acid residues of the abovementioned binding sites, a site of binding  $\text{TiO}_2$  to the cholinoreceptor becomes the most relevant one. The analysis of the amino acid composition of binding sites for each of the abovementioned substances demonstrates that polar uncharged amino acid residues Tyr83 and Asn419, as well as polar amino acid residue Glu172 are involved in sites of binding a  $\text{TiO}_2$  nanoparticle to the allosteric modulator LY2119620. As stated above, the latter affects the affinity of the receptor to the orthosteric ligands and may directly activate G-proteins as allosteric agonists. At the same time, the modulation of the binding site for the allosteric modulator with nanosized  $\text{TiO}_2$  due to the formation of bonds with amino acid residues, common for both sites, is likely to become a reason of the impairment of the coordinated regulation of conformational transformations in structure of the cholinoreceptor, induced by LY2119620 leading to an impairment of its function. A structure of the acetylcholine binding site with the muscarinic M2 receptor, in contrast to the structure of the binding sites of the  $\text{TiO}_2$  nanoparticle, the alosteric modulator LY2119620 and the orthosteric agonist ipexoro, is constructed according to another principle. There is no clear correlation between nonpolar and polar charged amino acid residues. Also, the binding sites of  $\text{TiO}_2$  and the acetylcholine do not contain common amino acid residues of the binding.

A molecular docking of the acetylcholine to an extracellular part of the muscarinic M3 cholinoreceptor using the PatchDock web server showed that the binding site and its environment include the following amino acid residues: Asp147, Tyr148, Ser151, Asn152, Ala238, Trp503, Tyr506, Tyr529, Cys532 (Fig. 11). The Geometric complementarity Score for this site was 3104, the Approximate interface area for the complex of muscarinic M3 acetylcholine receptor and acetylcholine was  $336.70 \text{ \AA}^2$ , and the Atomic Contact Energy ACE was (-117.62).



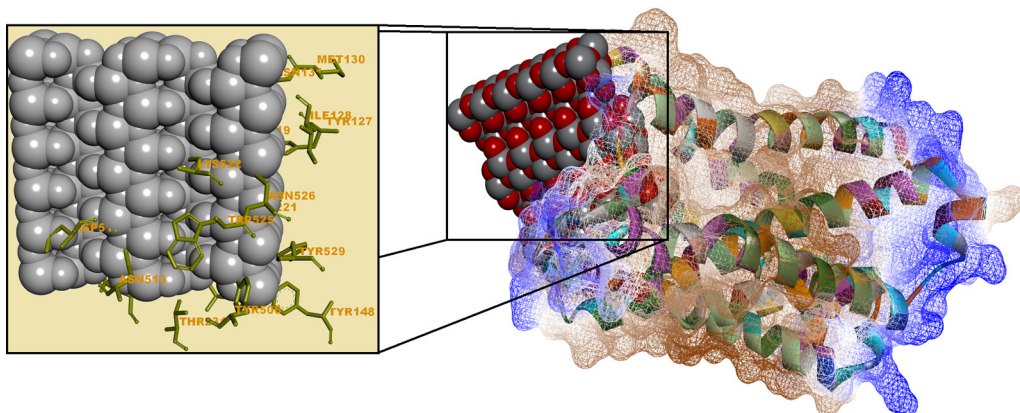


**Fig. 11.** The binding site of the acetylcholine (PubChem CID: 187) to the M3 type muscarinic acetylcholine receptor (Score = 3104, Area = 336.70 Å<sup>2</sup>, ACE = -117.62)

**Рис. 11.** Сайт зв'язування агоніста ацетилхоліну (PubChem CID: 187) з мускариновим ацетилхоліновим рецептором М3 типу (Score = 3104, Area = 336.70 Å<sup>2</sup>, ACE = -117.62)

Among the amino acids of the site are nonpolar (Ala238, Trp503), polar uncharged (Tyr148, Ser151, Asn152, Tyr506, Tyr529, Cys532) and polar charged (Asp147) amino acid residues. They are in the ratios of 0.22:0.67:0.11 and corresponding a distribution of the Gibbs free energy (kJ/mol): (-1.22); (-1.2); (-0.15).

The amino acid composition and environment of the TiO<sub>2</sub> binding site with the M3 muscarinic acetylcholine receptor that showed the highest binding affinity (Fig.12), includes such amino acid residues as: Tyr127, Ile128, Met130, Asn131, Tyr148, Glu219, Phe221, Leu225, Ser226, Glu227, Pro228, Thr231, Tyr506, Asn513, Asp517, Lys522, Trp525, Asn526, Tyr529. The Geometric shape complementarity score for this site was acquired a value of 9284, the Approximate interface area of the receptor complex and nanoparticle was 1368.10 Å<sup>2</sup>, Atomic Contact energy was (260.01).



**Fig. 12.** A site of binding the TiO<sub>2</sub> nanoparticle to the muscarinic acetylcholine M3 type receptor (Score = 9284, Area = 1368.10 Å<sup>2</sup>, ACE = 260.01)

**Рис. 12.** Сайт зв'язування наночастинки діоксиду титану з мускариновим ацетилхоліновим рецептором М3 типу (Score = 9284, Area = 1368,10 Å<sup>2</sup>, ACE = 260,01)

It was established that a binding site of the  $\text{TiO}_2$  nanoparticle to the muscarinic acetylcholine M3 type receptor, in contrast to the M2 type receptor, forms not four, but three groups of consecutively located amino acid residues. The first group of amino acids includes Tyr127, Ile128, Met130, Asn131; the second group of amino acids includes Glu219, Phe221, Leu225, Ser226, Glu227, Pro228, Thr231; the third group of amino acids includes Asn513, Asp517, Lys522, Trp525, Asn526, Tyr529; and two amino acid residues not belonging to the group includes Tyr148, Tyr506. The distances between them are 88 and 282 amino acid residues, respectively. Among the amino acids of the site are non-polar amino acid residues (Ile128, Met130, Phe221, Leu225, Pro228, Trp525), polar uncharged (Tyr127, Asn131, Tyr148, Ser226, Thr231, Tyr506, Asn513, Asn526, Tyr529), polar charged (Glu219, Glu227, Asp517, Lys522) residues that are in the ratios of 0,32:0,47:0,21 and the corresponding distribution of Gibbs free energy (kJ/mol) is (0,79); (-1,43); (-1,34).

Comparison of the binding sites of the nanosized  $\text{TiO}_2$  and the acetylcholine with the muscarinic M3 type receptor has shown that the polar uncharged amino acid residues such as Tyr148, Tyr506 and Tyr529 are included into the binding sites both  $\text{TiO}_2$  nanoparticle and acetylcholine.

Therefore, the results of studies on the spontaneous contractive activity of circular stomach SM of rats antrum conducted using a tenzometric method demonstrated that  $\text{TiO}_2$  suspensions with sizes of the nanoparticles of (4–8) nm and (1–3) nm change the structure of its contractive cycles. In a final result, there is a decrease in the total efficiency of the contractions-relaxations of muscle preparations compared to the control (which is proven by the decrease in MU and AU indices) with a better expressed inhibiting impact at the effect of  $\text{TiO}_2$  of (4–8) nm. There were also changes in the kinetic parameters of high potassium contraction of smooth muscles at the effect of  $\text{TiO}_2$ : a decrease in the velocity of contractions occurs at the effect of  $\text{TiO}_2$  suspension with a size of nanoparticles of (4–8) nm, whereas the application of  $\text{TiO}_2$  suspension with the size of nanoparticles of (1–3) nm leads to their increase compared to the control. Calculations of the ratio of velocities of the contractions-relaxations of SM at the effect of  $\text{TiO}_2$  (4–8) nm demonstrated that this parameter remained in a range of the control. In these conditions, an impairment of the coordination processes between  $V_{nc}$  and  $V_{nr}$  occurred at the effect of  $\text{TiO}_2$  suspension with a size of nanoparticles of (1–3) nm.  $\text{TiO}_2$  ((4–8) nm and (1–3) nm) also had a modulating effect on the cholinergic excitation of smooth muscles, accompanied with an increase in the normalized maximal velocity of their contraction, induced by acetylcholine: the ratio of velocities of contractions-relaxations remained in the range of the control at the effect of  $\text{TiO}_2$  suspension (1–3) nm, whereas its considerable increase compared to a control occurred at the effect of this nanosized material of (4–8) nm. In all the conditions of experiments the level of muscle tone remained unchanged. The molecular docking of  $\text{TiO}_2$  nanoparticle to the extracellular part of a muscarinic choline M2 type receptor demonstrated a possibility of forming the bonds with some amino acids of the site of its allosteric modulator that impacts an affinity of this receptor to the orthosteric ligands. The acetylcholine and  $\text{TiO}_2$  do not compete for binding sites that may be related, firstly, to a fact that  $\text{TiO}_2$  cannot penetrate to the acetylcholine binding site due to a discrepancy between sizes of the nanoparticle and the acetylcholine binding site (the dimensions of the  $\text{TiO}_2$  nanoparticle are  $(18.925 \times 3.785 \times 19.028) \text{ \AA}^3$ , whereas acetylcholine is  $(7.214 \times 2.485) \text{ \AA}$ ), and secondly,  $\text{TiO}_2$  can block the entry of

the agonist into its binding site. Concerning the muscarinic M3 type cholinergic receptor, there may be competitive relationships between TiO<sub>2</sub> and acetylcholine for binding within the site boundaries.

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## ВПЛИВ НАНО-TiO<sub>2</sub> НА ФУНКЦІОНУВАННЯ ГЛАДЕНЬКИХ М'ЯЗІВ ШЛУНКА: ДОСЛІДЖЕННЯ *IN VITRO* ТА *IN SILICO*

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Нанорозмірні матеріали, зокрема, наночастинки TiO<sub>2</sub>, розміром менше 10 нм є системами, що мають надлишкову енергію та високу хімічну активність, а наночастинки близько (1–3) нм практично без енергії активації вступають у реакції з іншими хімічними сполуками, що спричиняє утворення речовин із новими властивостями. Енергію, яку запасають такі об'єкти, визначають насамперед некомпенсованістю зв'язків поверхневих і приповерхневих атомів, що є причиною виникнення поверхневих явищ. У зв'язку з цим нами було здійснено дослідження *in vitro* та *in silico* дії нано-TiO<sub>2</sub> з розміром наночастинок (1–3) нм та (4–8) нм на функціонування гладеньких м'язів шлунка щурів.

Тензометричним методом в ізометричному режимі встановлено, що суспензії TiO<sub>2</sub> з розмірами наночастинок (4–8) та (1–3) нм змінюють структуру скорочуваль-



них циклів спонтанних скорочень кільцевих гладеньких м'язів антрального відділу шлунка щурів зі зниженням їхньої сумарної ефективності (зменшенням скоротливих індексів Монтевідео та Олександрійських одиниць). За таких умов змінюються також кінетичні параметри гіперкалієвої контрактури та скорочень, індукованих медіатором ацетилхолінових рецепторів гладеньких м'язів – ацетилхоліном з порушенням процесів узгодження між швидкістю скорочення та розслаблення більш виражених за дії діоксиду титану у першому випадку (1–3) нм, а у другому – (4–8) нм. Проведеним молекулярним докінгом наночастинки  $TiO_2$  з екстрацелюлярною частиною мускаринового холінорецептора M2 типу встановлено можливість утворення зв'язків з певними амінокислотами сайту його алостеричного модулятора, що впливає на спорідненість цього рецептора до ортостеричних лігандів. За своїм амінокислотним складом сайт зв'язування  $TiO_2$  не конкурує за місця зв'язування медіатора цього типу рецепторів ацетилхоліну. Докінгом  $TiO_2$  з мускариновим холінорецептором M3 типу виявлено, що наявні спільні амінокислотні залишки як для наночастинки, так і для ацетилхоліну, з якими утворюються зв'язки у ортостеричному сайті зв'язування. Це доводить про те, що в цьому сайті зв'язування можуть мати місце конкурентні відносини за місця зв'язування діоксиду титану й ацетилхоліну в межах сайту.

**Ключові слова:** тензометричний метод, кільцеві гладенькі м'язи, діоксид титану, молекулярний докінг, мускаринові ацетилхолінові рецептори

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