DOI: 10.26693/jmbs05.06.370

UDC 57.084.1+57.085.1+57.085.2+57.087.1+616-092

Stetska V. O., Moroz O. F., Dovbynychuk T. V., Tolstanova G. M., Zholos A. V.

The Role of TRPV4 Cation Channels in Smooth Muscle Contractile Activity in Rats

Taras Shevchenko National University, Kyiv, Ukraine

a.zholos@univ.net.ua

Although it was shown that transient receptor potential channels are expressed in the intestinal and myometrial smooth muscle cells and can control gastrointestinal motility and regulate uterine contractility the specific role of transient receptor potential vanilloid-type 4 channel in smooth muscle cells contraction remain largely unknown.

The purpose of the study was to test the action of transient receptor potential vanilloid-type 4 selective agonist GSK1016790A on smooth muscle cells contraction in rat's colon with experimental Parkinson's disease and in the pregnant rat uterus (18-22 days of gestation).

Material and methods. The Parkinson's disease was induced by single unilateral stereotaxic injection of 12 µg 6-OHDA. The percentage of destroyed dopaminergic neurons was evaluated in apomorphine test (0.5 mg/kg, i.p.) at 1 and 2 weeks after surgery. The water content in faeces was evaluated on the 1st day, then at the 3rd week and 7th month of the experiment. The daily volume of water consumption and gastrointestinal transit time were evaluated at the 3rd week and 7th month after surgery. The action of transient receptor potential vanilloid-type 4 agonist GSK1016790A (0.3 mmol) on smooth muscle cells of colon and myometrium strips contraction was estimated by isometric tension recording.

Results and discussion. The apomorphine test showed a progressive increase in the number of turns between the 1st and 2nd week after inducing 6-OHDA-PD. The water content in faeces was increased at the 3rd week ($P<0.05$) vs. 1st day of the experiment. The rats with 6-OHDA-PD drank less water vs. placebo and intact groups. We observed a 17% delayed GI transit time in 6-OHDA-PD rats ($P<0.01$) vs. intact and 21% vs. sham-lesioned group of rats 3 weeks after the 6-OHDA treatment. 7 months after the surgery GI transit time was increased more than twice in all studied groups. Transient receptor potential vanilloid-type 4 agonist action on smooth muscle cells of 6-OHDA-PD rats was reduced by 21% compared to intact group and by 46% in sham-lesioned group ($P<0.05$). After the application of GSK1016790A the rat myometrium strips a 28.4% ($P<0.05$) decrease of the contractile force was recorded. It was accompanied by a 30.7% ($P<0.05$) decline of the muscle work estimated as the area under the contractile curve. Suppression of the amplitude of uterine contraction was also followed by a 39.7% ($P<0.05$) decline of the

rise time constant of peaks but unchanged peak duration at the half maximal amplitude.

Conclusion. We conclude that pharmacological activation of transient receptor potential vanilloid-type 4 ion channels by their selective agonist GSK1016790A decreased the contractile activity of both colon smooth muscle cells in Parkinson's disease rats' model and the myometrium in pregnant rats.

Keywords: transient receptor potential vanilloid-type 4 ion channels, gastrointestinal smooth muscle cells, pregnant myometrium contraction, Parkinson's disease.

Research relation to the programs, plans, and department themes. This work was supported by the Ministry of Education and Science of Ukraine [grant No. 19BF036-01].

Introduction. Transient receptor potential (TRP) channels belong to the superfamily of cationic channels that change membrane potential or intracellular calcium concentration ($[Ca^{2+}]_i$) and can be involved in different pathological mechanisms, mechanosensation [1, 2], pain transduction [3], regulation of gastrointestinal (GI) motility [4, 5] among many other important functions. Intracellular Ca^{2+} is critical for smooth muscle cells (SMCs) contractile response development and regulation [6]. GI motility depends on the contractile activity of the SMCs of the tunica muscularis [7]. Furthermore, disruption of Ca^{2+} homeostasis in neuronal cells results in decreased neuronal functions and neurodegenerative diseases such as Parkinson's disease (PD) [8-10].

Although the TRP family genes are expressed in the intestine and can control GI motility, the specific role of transient receptor potential vanilloid-type 4 (TRPV4) in this process remains largely unknown. TRPV4 cation channel is widely expressed in epithelial cells, vascular endothelium, lung, chondrocytes and neurons [4, 11].

The upregulation of TRPV4 in vascular endothelial cells contributes to the progression of colonic inflammation via an increase in vascular permeability [12]. Injection of TRPV4 agonist GSK1016790 induced hippocampal neuronal death [13]. Agonist of TRPV4 increases the intracellular Ca^{2+} level and the production of superoxide in lung macrophages [14].

Luo *et al.* [4] showed that TRPV4 can promote GI motility and can affect the function of intestinal muscularis macrophages (MMs). Moreover, this research

showed that TRPV4-responsive MMs mediated contractility by direct interaction with SMCs, independently of neuronal input. However, whether TRPV4 regulates GI motility in PD remains to be explored.

TRPV4 has been found to be expressed in myometrium SMCs where this channel is involved in the regulation of uterine contractility [15]. In the murine model of preterm labor inhibition of TRPV4 with its selective antagonist appeared to prolong pregnancy [16] implying that TRPV4 contributes to myometrium contractility. However, taking into account the well-characterized colocalization of these channels with calcium-dependent potassium channels and voltage-gated L-type Ca^{2+} channels the cellular response to activation of TRPV4 could be also of the inhibitory type [17].

The purpose of this study was to test the action of TRPV4 selective agonist GSK1016790A on SMCs contraction in rat's colon with PD and in the pregnant rat uterus.

Material and methods. *Animals.* Male Wistar rats (220-250 g, $n=34$), pregnant rats (18-22 days of gestation, $n=10$) were bred and housed under standard conditions of temperature (21-23 °C), lighting (12/12 h light-dark cycle), and humidity (30-35%). The study design was approved by the Bioethical Committee of the ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine.

Modeling of PD. The unilateral dopamine deficiency in rats was performed by disruption of dopaminergic neurons (DA) with a unilateral lesion of the medial forebrain bundle (coordinates (mm) from the bregma (AP = -2,2; ML = 1,5; DV = 8,8)) by stereotaxic microinjections of 12 μg of the selective neurotoxin 6-hydroxydopamine (6-OHDA) (Sigma, USA) [18]. The sham-lesioned group of animals was injected with 4 μl 0.9% sterile NaCl. The percentage of destroyed dopaminergic neurons was evaluated by the apomorphine test (0.5 mg/kg, *i.p.*) at 1 and 2 weeks after surgery.

Gastrointestinal (GI) Transit Assay. Rats were orally gavaged with 0.5 ml aqueous solution of 3% carmine red (ECO Resource, Ukraine) and placed in a new cage with no bedding. The time at which gavage took place was recorded as T_0 . Total GI transit time was considered as the interval between T_0 and the time of the first observance of carmine red in stool [19].

The Water Intake and Water Content in Faeces. The freshly expelled faeces of rats were collected and weighted (m_w – wet weight). It was dried in an incubator at $t=60$ °C for 24 h (m_d – dry weight). The calculation of water content (W, %) was carried out according to the formula: $W = 100 - (m_d * 100 \% / m_w)$. To determine water intake of rats, each animal was transferred into a separate cage for 3 days (adaptation period for new conditions). The volume of drank water was measured each 24 h during 7 days, in

3 weeks and 7 months after surgery. The volume of drank water/per day for each animal was averaged per group.

Isometric Tension Recording of Colon Strips. The longitudinal muscle layers of rats' colon were peeled off and placed in a modified Krebs solution containing (in mM): 120 NaCl, 12 glucose, 6 KCl, 15.5 NaHCO_3 , 1.2 $\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$, 1.2 $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 2.5 CaCl_2 , (pH=7.4). The tissue was cut into pieces (10x5 mm length) and placed in bath with the modified Krebs solution at 37°C for 40 min (adaptation period). Test stimulation to activate SMCs submaximal contraction was done by applying 80 mM KCl (for 1-2 min until the peak was clearly reached and immediately changed to the modified Krebs solution). The strips were then washed after KCl for 15 min and the KCl test was repeated. The role of TRPV4 in smooth muscle colon strips was determined by applying the selective agonist of TRPV4 channels GSK1016790A (Sigma, USA). The strips were perfused with solution containing 0.3 mmol/L GSK1016790A for 5 min, washed for 10 min in fresh Krebs solution, and the TRPV4 agonist was applied for the second time.

Isometric Tension Recording of Myometrial Strips. Circular layer of the myometrium was cleaved of the endometrium, cut into small strips (10 mm*3mm) and mounted into the organ bath on metal hooks, one of which was connected to the capacitive force transducer and the other one was connected to the micromanipulator to set the 1.5 g passive resting tension. After the setting of 1.5 g of passive tension strips were equilibrated during 45 min and then control spontaneous contractions were recorded during 15 min. All pharmacological agents were added to the external solution directly into the bath and their effect was recorded during 5-7 min. The composition of the physiological salt solution used both for the strips' preparation and tensiometry experiments was (in mmol/l): NaCl – 133, NaHCO_3 – 16.3, NaH_2PO_4 – 1.38, KCl – 4.7, MgCl_2 – 1.05, glucose – 11.5, CaCl_2 – 2.73, HEPES - 10 (pH=7.4).

Statistical analysis. Data are presented as mean $\pm$ standard error of the mean (SEM). Homogeneity of variance was assessed using the Levene test. Statistical analysis of the data was performed using Kruskal-Wallis test or one-way analysis of variance (ANOVA) followed by the Fisher LSD post hoc test. The difference was considered statistically significant at $P < 0.05$. GraphPad Prism 8 was used for making graphs.

Results and discussion. The number of apomorphine-induced turns confirmed destroyed DA neurons in the midbrain. On the 8th day of the experiment, 6-OHDA rats made 68.22 ± 15.09 turns/30 min in apomorphine test. At the 2nd week the number of turns increased to 113.22 ± 20.86 turns/30 min. Statistical significance was not observed (**Fig. 1A**). The number

of turns of less than 100 turns/30 min corresponds to less than 64% destroyed DA neurons [18].

In our study, we observed a 17% delayed GI transit time in 6-OHDA-PD rats ($P < 0.01$) vs. intact and 21% vs. sham-lesioned group of rats at 3 weeks of the experiment (Fig. 1B). At 7 months after the surgery GI transit time was increased more than twice in rats of all studied groups compared to those at 3 weeks of the experiment, while 6-OHDA-PD rats still had delayed GI transit vs. both control groups ($P < 0.01$).

We observed the decrease in water intake volume at the 3rd week of the experiment in 6-OHDA-PD rats (25.04 ± 5.22 ml) vs. intact (30.10 ± 4.85 ml) ($P < 0.05$) and sham-lesioned (30.92 ± 10.04 ml) groups of rats (Fig. 1C). The volume of water intake after 7 months increased in all groups compared to that after 3 weeks to 33.40 ± 0.97 ml in the intact group, 35.11 ± 1.96 ml in the sham-lesioned group and 32.3 ± 1.19 ml in 6-OHDA-PD rats ($P < 0.05$). Water content in rat faeces was increased ($P < 0.05$) after 3 weeks of experiment in all experimental groups (Fig. 1D). Water content in faeces in the 6-OHDA-PD rats was lower during all experimental period vs. sham-lesioned group, that might demonstrate a decrease of colonic motility. At 3 weeks after surgery, the delayed GI transit in 6-OHDA-PD rats correlated with the decrease in faecal water content and the water in-

take volume. At 7 months, we observed the physiological adaptation and no significant difference in these parameters between the studied groups.

GI motility results from coordinated contractions of the tunica muscularis, the muscular layer of the GI tract [20]. Besides GI transit examination, to explore the role of TRPV4 in colon motility, we recorded isometric tension of the colon smooth muscle strips of 6-OHDA-PD rats 7 months after surgery. In smooth muscle tissue, the KCl-induced contraction was used to study excitation-contraction coupling and as a standard contraction response for the evaluation of the effect of different substances on smooth muscle contractility [21]. KCl-induced contraction causes strong membrane depolarization, openings of L-type voltage-gated Ca^{2+} channels, Ca^{2+} influx and, ultimately, activation of Ca^{2+} -dependent myosin light chain (MLC) kinase, that results in MLC phosphorylation [22]. The regulatory light chain (RLC) of myosin initiates a smooth muscle contraction that is determined by the activities of MLC kinase and the phosphatases, which phosphorylate and dephosphorylate the RLC, respectively [23]. The maximum contractile activity of SMCs after activation by 80 mM KCl was reduced in 6-OHDA-PD rats to 2.13 ± 1.44 g that is 16% less than that observed in sham-lesioned group and 60% less than in the intact group (Fig. 1E). Xiao-Lin Xiu *et al.*

Figure 1. Decreased colonic motor activity in rats with 6-OHDA-PD vs. the control groups: (A) The apomorphine-induced rotational behavior in rats at the 1st and 2nd weeks after 6-OHDA-induced PD, $n = 9$; (B) The total gastrointestinal (GI) transit time (by 0.5 ml of 3% carmine red), $n = 34$; (C) The volume of water consumption (ml per rat), $n = 30$; (D) The water content in faeces, $n = 34$; (E) The maximum peak of contraction after 80 mM KCl stimulation of smooth muscle colon strips (g), $N=16$, $n = 32$; (F) The effect of TRPV4 agonist GSK1016790A on smooth muscle colon strips contraction, %, $N=16$, $n = 32$; $*P < 0.05$, $**P < 0.01$ ($M \pm SEM$)

[24] have shown that maximum amplitude of phasic contraction induced by carbachol in gastric SMCs of the 6-OHDA rats was decreased approximately by 60% compared to control.

GSK1016790A is the selective and potent agonist of TRPV4 and it is widely used to study TRPV4 physiological function *in vitro* and *in vivo* [11]. Fichna *et al* showed that the TRPV4 agonist GSK1016790A reduced colonic contractility and increased inhibitory neurotransmission *in vitro*. *In vivo*, TRPV4 activation slowed GI motility and reduced stool production in mice [25].

We observed a 90% increase of contraction of colon smooth muscle strips in sham-lesioned PD rats after application of TRPV4 agonist GSK1016790A compared to the intact group ($P < 0.05$) (Fig. 1G). Agonist action on SMCs of 6-OHDA-PD rats was by 21% lower than that in the intact group and by 46% in sham-lesioned group ($P < 0.05$). In the present study, we report for the first time the effect of GSK1016790A on colonic motility in rats with 6-OHDA-induced PD *in vivo* and *in vitro*.

Bain and Mowat [26] and De Schepper *et al.* [27] have demonstrated the interaction between SMCs in the muscularis layers and macrophages and that this cooperation may regulate GI motility. MMs can regulate GI motility during inflammation through se-

cretion of inflammatory cytokines [28, 29]. MMs can regulate GI motility due to growth factor BMP2 when the intestine is not inflamed. But the role of BMP2 in regulating GI motility during inflammation remains to be determined [30]. Luo *et al.* have shown that the MMs-specific expression of TRPV4 directly regulated the function of intestinal SMCs to control GI motility [4]. In addition, reduced intestinal motility can be connected with prostaglandin synthesis. Nitric oxide and prostaglandin E2 (PGE2) are the primary inflammatory mediators released by activated MMs that regulate GI motility [31]. Prostaglandin F2 α (PGF2 α) is the endothelium-derived contracting factor (EDCFs) induced by TRPV4 activation [32]. It was also shown that GSK1016790A or acetylcholine triggered the release of PGF2 α (prostaglandin F2 α) that was reduced by inhibition of PKC or ERK1/2 [33].

To reveal some characteristic features of the effect of TRPV4 activation on SMCs contractility we studied the response of uterine smooth muscle tissue to the selective agonist of these channels. After the application of GSK1016790A to rat myometrium strips a 28,4% ($P < 0.05$) decrease of the contractile force was measured. It was accompanied by a 30,7% ($P < 0.05$) reduction of muscle force that was expressed as the area under the curve (Fig. 2. A, B).

Figure 2. Decrease of the myometrium contractility in rats under pharmacological activation of TRPV4: (A) Amplitude of spontaneous contractions and area under the curve (AUC) in response to the selective TRPV4 agonist GSK1016790A (0.3 μ M, $n = 8$); (B) Typical record of the uterine strips spontaneous and GSK1016790A-induced contractions; (C) Duration of contraction peaks at half maximal amplitude does not change in response to TRPV4 agonist although the rise time constant (τ) is reduced, $n = 8$; (D) Linear correlation between work of the muscle (AUC) and peak contractile force during TRPV4 activation, $n = 10$; * $P < 0.05$

Suppression of the amplitude of uterine contractions was accompanied by a 39.7% ($P < 0.05$) decrease of the rise time constant (rise tau) of peaks but unchanged peak duration at the half maximal amplitude (**Fig. 2C**). Investigation of the relative contribution of the contractile force and peak duration to the work performed by the muscle strip revealed that the AUC changed linearly with the peak amplitude (**Fig. 2D**). Our results that are at odds with those obtained on murine myometrium might demonstrate the possible interactions of BK_{Ca} channels and TRPV4 when calcium entering SMCs causes the opening of potassium channels that alleviate contractions via membrane hyperpolarization [34]. Reduction of contractility during the activation of TRPV4 has been also demonstrated in the urinary bladder strips where the functional

interaction with BK_{Ca} channels was also suggested; however, further investigations need to be performed [35].

Conclusion and prospects of further researchers. In conclusion, pharmacological activation of TRPV4 cation channels by their selective agonist GSK1016790A decreased the contractile activity of colon SMCs in PD rats model and suppressed contractions of the myometrium in pregnant rats. TRPV4 may thus represent a promising therapeutic target for the correction of GI motility disorders in PD pathogenesis. The mechanisms of TRPV4 involvement in the regulation of uterine contractility require further investigations but it also appears to be promising in the context of the link between procontractile and relaxing pharmacological agents.

References

1. Samanta A, Hughes TET, Moiseenkova-Bell VY. Transient receptor potential (TRP) channels. *Subcellular Biochemistry*. 2018; 87: 141-165. doi: 10.1007/978-981-10-7757-9_6
2. Michalick L, Kuebler WM. TRPV4-A Missing Link Between Mechanosensation and Immunity. *Front. Immunol.* 2020; 11: 413. doi: 10.3389/fimmu.2020.00413
3. Moore C, Gupta R, Jordt SE, Chen Y, Liedtke W.B. Regulation of Pain and Itch by TRP Channels. *Neurosci Bull.* 2018; 34: 120-142. doi: 10.1007/s12264-017-0200-8
4. Luo J, Qian A, Oetjen LK, Yu W, Yang P, Feng J, et al. TRPV4 Channel Signaling in Macrophages Promotes Gastrointestinal Motility via Direct Effects on Smooth Muscle Cells. *Immunity*. 2018; 49: 107-119. doi: 10.1016/j.immuni.2018.04.021
5. Cenac N, Bautzova T, Le Faouder P, Veldhuis NA, Poole DP, Rolland C, et al. Quantification and potential functions of endogenous agonists of transient receptor potential channels in patients with irritable bowel syndrome. *Gastroenterology*. 2015; 149: 433-444. doi: 10.1053/j.gastro.2015.04.011
6. Yamazawa T, Iino M. Simultaneous imaging of Ca^{2+} signals in interstitial cells of Cajal and longitudinal smooth muscle cells during rhythmic activity in mouse ileum. *J Physiol.* 2002; 538: 823-835. doi: 10.1113/jphysiol.2001.013045
7. Perrino BA. Regulation of gastrointestinal motility by Ca^{2+} /calmodulin-stimulated protein kinase II. *Arch. Biochem Biophys.* 2011; 510: 174-181. doi: 10.1016/j.abb.2011.03.009
8. Bollimuntha S, Pani B, Singh BB. Neurological and motor disorders: Neuronal store-operated Ca^{2+} signaling: An overview and its function. *Adv Exp Med Biol.* 2017; 993: 535-556. doi: 10.1007/978-3-319-57732-6_27
9. Sukumaran P, Sun Y, Schaar A, Selvaraj S, Singh BB. TRPC channels and Parkinson's disease. *Adv Exp Med Biol.* 2017; 976: 85-94. doi: 10.1007/978-94-024-1088-4_8
10. Cali T, Ottolini D, Brini M. Mitochondria, calcium, and endoplasmic reticulum stress in Parkinson's disease. *BioFactors*. 2011; 37: 228-240. doi: 10.1002/biof.159
11. Baratchi S, Keov P, Darby WG, Lai A, Khoshmanesh K, Thurgood P, et al. The TRPV4 agonist GSK1016790A regulates the membrane expression of TRPV4 channels. *Front Pharmacol.* 2019; 9. doi: 10.3389/fphar.2019.00006
12. Matsumoto K, Kato S. Physiological and pathophysiological roles of trpv4 channel in gastrointestinal tract. *Folia Pharmacol Jpn.* 2019; 154: 92-96. doi: 10.1254/fpj.154.92
13. Jie P, Lu Z, Hong Z, Li L, Zhou L, Li Y, et al. Activation of Transient Receptor Potential Vanilloid 4 is Involved in Neuronal Injury in Middle Cerebral Artery Occlusion in Mice. *Mol Neurobiol.* 2016; 53: 8-17. doi: 10.1007/s12035-014-8992-2
14. Hamanaka Y, Park D, Yin P, Annangudi SP, Edwards TN, Sweedler J, et al. Transcriptional Orchestration of the Regulated Secretory Pathway in Neurons by the bHLH protein DIMM. *Curr Biol.* 2010; 20: 9-18. doi: 10.1016/j.cub.2009.11.065
15. Singh V, Ram M, Kandasamy K, Thangamalai R, Choudhary S, Das, JR, et al. Molecular and functional characterization of TRPV4 channels in pregnant and nonpregnant mouse uterus. *Life Sci.* 2015; 122: 51-58. doi: 10.1016/j.lfs.2014.12.010
16. Ying L, Becard M, Lyell D, Han X, Shortliffe L, Husted CI, et al. The transient receptor potential vanilloid 4 channel modulates uterine tone during pregnancy. *Sci Transl Med.* 2015; 7(319): 319ra204. doi: 10.1126/scitranslmed.aad0376

17. White JPM, Cibelli M, Urban L, Nilius B, McGeown JG, Nagy I. TRPV4: Molecular Conductor of a Diverse Orchestra. *Physiol Rev.* 2016; 96: 911-973. doi: 10.1152/physrev.00016.2015
18. Talanov SA, Oleshko NN, Tkachenko MN, Sagach VF. Pharmacoprotective Influences on Different Links of the Mechanism Underlying 6-Hydroxydopamine-Induced Degeneration of Nigro-Striatal Dopaminergic Neurons. *Neurophysiology.* 2006; 38: 128-133. doi: 10.1007/s11062-006-0035-9
19. Yano JM, Yu K, Mazmanian SK, Correspondence EYH, Donaldson GP, Shastri GG, et al. Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis Article Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. *Cell.* 2015; 161: 264-276. doi: 10.1016/j.cell.2015.02.047
20. Sanders KM, Koh SD, Ro S, Ward SM. Regulation of gastrointestinal motility-insights from smooth muscle biology. *Nat Rev Gastroenterol Hepatol.* 2012; 9: 633-645. doi: 10.1038/nrgastro.2012.168
21. Karaki H, Urakawa N, Kutsy P. Potassium-induced contraction in smooth muscle. *Japanese J Smooth Muscle Res.* 1984; 20: 427-444. doi: 10.1540/jsmr1965.20.427
22. Ratz PH, Berg KM, Urban NH, Miner AS. Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. *Am J Physiol. Cell Physiol.* 2005; 288. doi: 10.1152/ajpcell.00529.2004
23. Malmqvist U, Arner A. Kinetics of contraction in depolarized smooth muscle from guinea-pig taenia coli after photodestruction of nifedipine. *J Physiol.* 1999; 519: 213-221. doi: 10.1111/j.1469-7793.1999.02130.x
24. Xiu XL, Zheng LF, Liu XY, Fan YY, Zhu JX. Gastric smooth muscle cells manifest an abnormal phenotype in Parkinson's disease rats with gastric dysmotility. *Cell Tissue Res.* 2020; 381: 217-227. doi: 10.1007/s00441-020-03214-9
25. Fichna J, Poole DP, Veldhuis N, MacEachern SJ, Saur D, Zakrzewski PK, et al. Transient receptor potential vanilloid 4 inhibits mouse colonic motility by activating NO-dependent enteric neurotransmission. *J Mol Med.* 2015; 93: 1297-1309. doi: 10.1007/s00109-015-1336-5
26. Bain CC, Mowat AM. Macrophages in intestinal homeostasis and inflammation. *Immunol Rev.* 2014; 260: 102-117. doi: 10.1111/imr.12192
27. De Schepper S, Verheijden S, Aguilera-Lizarraga J, Jones E, Lambrechts D, Boeckxstaens G, et al. Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis Article Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis. *Cell.* 2018; 175: 400-415. doi: 10.1016/j.cell.2018.07.048
28. Van Der Zanden EP, Boeckxstaens GE, De Jonge WJ. The vagus nerve as a modulator of intestinal inflammation. *Neurogastroenterol Motil.* 2009; 21: 6-17. doi: 10.1111/j.1365-2982.2008.01252.x
29. Mikkelsen HB. Interstitial cells of Cajal, macrophages and mast cells in the gut musculature: morphology, distribution, spatial and possible functional interactions. *J Cell Mol Med.* 2010; 14: 818-832. doi: 10.1111/j.1582-4934.2010.01025.x
30. Muller PA, Koscsó B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell.* 2014; 158: 300-313. doi: 10.1016/j.cell.2014.04.050
31. Mori D, Watanabe N, Kaminuma O, Murata T, Hiroi T, Ozaki H, et al. IL-17A induces hypo-contraction of intestinal smooth muscle via induction of iNOS in muscularis macrophages. *J Pharmacol Sci.* 2014; 125: 394-405. doi: 10.1254/jphs.14060FP
32. Zhang P, Sun C, Li H, Tang C, Kan H, Yang Z, et al. TRPV4 (Transient Receptor Potential Vanilloid 4) Mediates Endothelium-Dependent Contractions in the Aortas of Hypertensive Mice. *Hypertension.* 2018; 71: 134-142. doi: 10.1161/HYPERTENSIONAHA.117.09767
33. Li H, Kan H, He C, Zhang X, Yang Z, Jin J, et al. TRPV4 activates cytosolic phospholipase A2 via Ca²⁺-dependent PKC/ERK1/2 signalling in controlling hypertensive contraction. *Clin Exp Pharmacol Physiol.* 2018; 45: 908-915. doi: 10.1111/1440-1681.12959
34. Grace MS, Bonvini SJ, Belvisi MG, McIntyre P. Modulation of the TRPV4 ion channel as a therapeutic target for disease. *Pharmacol Ther.* 2017; 177: 9-22. doi: 10.1016/j.pharmthera.2017.02.019
35. Isogai A, Lee K, Mitsui R, Hashitani H. Functional coupling of TRPV4 channels and BK channels in regulating spontaneous contractions of the guinea pig urinary bladder. *Pflügers Arch. - Eur J Physiol.* 2016; 468: 1573-1585. doi: 10.1007/s00424-016-1863-0

УДК 57.084.1+57.085.1+57.085.2+57.087.1+616-092

РОЛЬ TRPV4 КАТІОННИХ КАНАЛІВ НА СКОРОТЛИВУ АКТИВНІСТЬ ГЛАДЕНЬКИХ М'ЯЗІВ ЩУРІВ

Стецька В. О., Мороз О. Ф., Довбинчук Т. В., Толстанова Г. М., Жолос О. В.

Резюме. Хоча було показано, що канали транзиторного рецепторного потенціалу експресуються в гладеньком'язових клітинах кишечника та міометрію, де вони можуть контролювати моторику шлунково-кишкового тракту та регулювати скорочення матки, але роль транзиторного рецепторного потенціалу ванілоїдного типу 4 у скороченні гладеньких м'язів кишечника залишається в основному невідомою.

Метою цього дослідження було перевірити дію селективного агоніста транзйентного рецепторного потенціалу ванілоїдного типу 4 GSK1016790A на скорочення гладеньких м'язів кишечника товстої кишки щурів із експериментальною хворобою Паркінсона та матки вагітних щурів (18-22 дні вагітності).

Хвороба Паркінсона викликала одноразовою односторонньою стереотаксичною ін'єкцією 12 мкг 6-OHDA. Відсоток зруйнованих дофамінергічних нейронів оцінювали апоморфіновим тестом (0,5 мг/кг, в/в) через 1 і 2 тижні після операції. Вміст води у фекаліях оцінювали на 1-й день, 3-й тиждень та 7-й місяць експерименту. Щоденний об'єм споживання води та час кишкового транзиту оцінювали на 3-й тиждень та 7-й місяць після операції. Дію агоніста транзйентного рецепторного потенціалу ванілоїдного типу 4 GSK1016790A (0,3 ммоль) на скорочення гладеньких м'язів кишечника смужок товстої кишки та міометрію оцінювали за допомогою ізометричного запису напруги.

Тест на апоморфін показав поступове збільшення кількості обертів між 1-м і 2-м тижнем після моделювання 6-OHDA-ХП. Вміст води у фекаліях збільшувався на 3-й тиждень ($P < 0,05$) у порівнянні з 1-м днем експерименту. Щури з 6-OHDA-ХП пили менше води у порівнянні з групами плацебо та інтактними. В результатах дослідження спостерігали на 17% затримку кишкового транзиту у щурів 6-OHDA-ХП ($P < 0,01$) у порівнянні з інтактними та на 21% у порівнянні з групою плацебо та 3 тижнем експерименту. Через 7 місяців після операції кишкового транзиту збільшився більш ніж удвічі у всіх досліджуваних групах. Дія агоніста на ГМК щурів з 6-OHDA-ХП була меншою на 21%, ніж у інтактній групі та на 46%, ніж у групі плацебо ($P < 0,05$). Після додавання GSK1016790A на смужки міометрію щурів було зафіксовано зниження скорочення на 28,4% ($P < 0,05$). Це супроводжувалось зменшеною на 30,7% ($P < 0,05$) роботою м'язу, яку розраховували як площа під кривою скорочення. Зниження амплітуди скорочень матки супроводжувалось зменшенням постійної часу зростання піків на 39,7% ($P < 0,05$) та незмінною тривалістю піку на рівні половини максимальної амплітуди.

Фармакологічна активація іонних каналів транзйентного рецепторного потенціалу ванілоїдного типу 4 їх селективним агоністом GSK1016790A знижувала скоротливу активність гладеньких м'язів кишечника товстої кишки у моделі ХП-щурів та пригнічувала скорочення міометрію у вагітних щурів.

Ключові слова: іонні канали транзйентного рецепторного потенціалу ванілоїдного типу 4, клітини гладеньких м'язів кишечника, скорочення міометрію вагітної, хвороба Паркінсона.

УДК 57.084.1+57.085.1+57.085.2+57.087.1+616-092

РОЛЬ TRPV4 КАТИОННЫХ КАНАЛОВ В СОКРАТИТЕЛЬНОЙ АКТИВНОСТИ ГЛАДКИХ МЫШЦ КРЫС

Стецкая В. О., Мороз О. Ф., Довбинчук Т. В., Толстанов А. Н., Жолос А. В.

Резюме. Хотя и было показано, что каналы транзйентного рецепторного потенциала экспрессируются в клетках гладкомышечных клеток кишечника и миометрия, где они могут контролировать моторику желудочно-кишечного тракта и регулировать сокращения матки, но роль транзйентного рецепторного потенциала ванилоидного типа 4 в сокращении гладкомышечных клеток остается в основном неизвестной.

Целью этого исследования было проверить действие селективного агониста транзйентного рецепторного потенциала ванилоидного типа 4 GSK1016790A на сокращение гладкомышечных клеток толстой кишки крыс с экспериментальной болезнью Паркинсона и в матке беременных крыс (срок беременности 18-22 дня).

Болезнь Паркинсона вызвали одноразовой односторонней стереотаксической инъекцией 12 мкг 6-OHDA. Процент разрушенных дофаминергических нейронов оценивали апоморфиновым тестом (0,5 мг/кг, в/в) через 1 и 2 недели после операции. Содержание воды в фекалиях оценивали на 1-й день, 3-ю неделю и 7-й месяц эксперимента. Ежедневный объем потребленной воды, время кишечного транзита оценивали на 3-ю неделю и 7-й месяц после операции. Действие агониста транзйентного рецепторного потенциала ванилоидного типа 4 GSK1016790A (0,3 ммоль) на сокращение гладкомышечных клеток полосок толстой кишки и миометрия оценивали с помощью изометрического записи напряжения.

Тест на апоморфин показал постепенное увеличение числа оборотов между 1-й и 2-й неделями после моделирования 6-OHDA-БП. Содержание воды в фекалиях увеличивалось на 3-ю неделю ($P < 0,05$) в сравнении с 1-м днем эксперимента. Крысы с 6-OHDA-БП пили меньше воды в сравнении с группами плацебо и интактными. Наблюдали на 17% задержку кишечного транзита у крыс 6-OHDA-БП ($P < 0,01$) в сравнении с интактными и на 21% в сравнении с группой плацебо и. 3 недель эксперимента. Через 7 месяцев после операции кишечного транзита увеличился более чем вдвое во всех исследуемых группах. Действие агониста на гладкие мышцы кишечника крыс с 6-OHDA-ХП была меньше на 21%, чем в интактной группе и на 46% в группе плацебо ($P < 0,05$). После добавления GSK1016790A на

полоски миометрия крыс было зафиксировано снижение сократительной силы на 28,4% ($P < 0,05$). Это сопровождалось уменьшенной на 30,72% ($P < 0,05$) работой мышцы, которую рассчитывали, как площадь под кривой. Снижение амплитуды сокращений матки сопровождалось уменьшением постоянной времени роста пиков на 39,7% ($P < 0,05$) и неизменной продолжительностью пика при половине максимальной амплитуды. Фармакологическая активация ионных каналов транзиентного рецепторного потенциала ванилоидного типа 4 их селективным агонистом GSK1016790A снижала сокращения гладкомышечных клеток толстой кишки в модели ХП-крыс и угнетала сокращение миометрия у беременных крыс.

Ключевые слова: ионные каналы транзиентного рецепторного потенциала ванилоидного типа 4, клетки гладких мышц кишечника, сокращение миометрия беременной, болезнь Паркинсона.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

Стаття надійшла 30.09.2020 р.

Рекомендована до друку на засіданні редакційної колегії після рецензування