

# Impact of a Newly Synthesized Molecule (2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide) on the Bioelectrogenesis and the Contractile Activity of Isolated Smooth Muscles

Vera N. Gledacheva<sup>1</sup>, Iliyana D. Stefanova<sup>1</sup>, Valeri I. Slavchev<sup>1</sup>, Rayna G. Ardasheva<sup>1</sup>,  
Atanas D. Kristev<sup>1</sup>, Stoyanka A. Nikolova<sup>2</sup>, Kremena E. Saracheva<sup>3</sup>, Darinka S. Dimitrova<sup>4</sup>

<sup>1</sup> Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University Plovdiv

<sup>2</sup> Department of Organic Chemistry, Faculty of Chemistry, University of Plovdiv

<sup>3</sup> Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University Plovdiv

<sup>4</sup> Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University Plovdiv

**Corresponding author:** Vera N. Gledacheva, Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, 15A Vassil Aprilov Blvd., 4002 Plovdiv, Bulgaria; E-mail: vera\_m@abv.bg; Tel.: +359 883 481 486

**Received:** 31 Oct 2019 ♦ **Accepted:** 25 Feb 2020 ♦ **Published:** 30 Sep 2020

**Citation:** Gledacheva VN, Stefanova ID, Slavchev VI, Ardasheva RG, Kristev AD, Nikolova SA, Saracheva KE, Dimitrova DS. Impact of a newly synthesized molecule (2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide) on the bioelectrogenesis and the contractile activity of isolated smooth muscles. *Folia Med (Plovdiv)* 2020;62(3):532-8. doi: 10.3897/folmed.62.e47844.

## Abstract

**Introduction:** Examination of the potential possibilities of 2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide (IQP) to affect bioelectrogenesis and the contractile activity of isolated smooth muscles (SM) from stomach.

**Aim:** Having in mind the structural similarities between the molecules of papaverine and IQP, the aim of the present study was to examine such features of the newly synthesized molecule that may potentially affect the muscle tonus, spontaneous bioelectrical and contractile activities of smooth muscles isolated from the stomach, basing on specific mechanisms of papaverine.

**Materials and methods:** The synthesis of IQP is based on the initially formed aziridine ring by principles of Gilbert's reaction. Impact of IQP on the bioelectrogenesis and the contractile activity of isolated smooth muscles from male Wistar rats was measured by the single sucrose-gap method and isometrically recorded.

**Results:** IQP ( $1 \times 10^{-5}$  –  $2.5 \times 10^{-4}$  mol/l) causes muscle relaxation, producing changes in two processes that have influence on the mechanical activity of smooth muscles:

1. Blocked  $\text{Ca}^{2+}$  influx through the potential-dependent membrane  $\text{Ca}^{2+}$  channels, followed in turn by lowering the  $\text{Ca}^{2+}$  intracellular levels. This effect is proved by the changes in the frequency and amplitude of spike-potentials in sucrose-bridge experiments when IQP is applied.
2. Activation of a cAMP-dependent signal cascade. The relaxing effect of IQP was significantly reduced in the presence of KT5720 ( $5 \times 10^{-6}$  mol/l), an inhibitor of protein kinase A.

**Conclusion:** We assume that there might be interconnections between these two IQP-dependent processes, because PKA-dependent phosphorylation of the L-type  $\text{Ca}^{2+}$  channels in smooth muscles provokes a reaction of inactivation.

## Keywords

biomechanical processes, gastric smooth muscles, isoquinoline skeleton, natural amino acids, new synthesis

## INTRODUCTION

It is always of a great interest to study the biological activity of newly synthesized molecules that show structural resemblance with medicaments, drugs or their derivatives. There is always a possibility to compare and detect similarities and differences in their structural and pharmacological properties, and hence detection of new physiological effects and intermolecular interactions. The most promising pathway in the search for new biologically active substances is based on the combinations of many pharmacological fragments determining exact pharmacological effects. Trying to evaluate the dependence structure-activity, we synthesized and evaluated a new isoquinoline: 2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide, labeled in our experiments as IQP. IQP belongs to the group of isoquinolines which comprises structures with interesting pharmacological properties.<sup>1-4</sup> IQP also possesses structural resemblance with papaverine.

Papaverine causes relaxation of smooth muscles. Its hypotensive effect is achieved through its negative influence on membrane, potential-dependent  $\text{Ca}^{2+}$  channels.<sup>5</sup> On the other hand, papaverine leads to an increase of the intracellular cyclic adenosine monophosphate (cAMP) levels through inhibition of phosphodiesterase activity.<sup>6-8</sup> All this makes papaverine a perfect instrument to cure smooth muscle spasms of the gastro-intestinal tract, gallbladder, kidneys, urethra<sup>9</sup>, blood vessels of the brain and heart<sup>9,10</sup>, as well as some cases of erectile dysfunction.<sup>11,12</sup>

## AIM

Having in mind the structural similarities between the molecules of papaverine and IQP, the aim of the present study was to examine such features of the newly synthesized molecule that may potentially affect the muscle tonus, spontaneous bioelectrical and contractile activities of smooth muscles isolated from the stomach, basing on specific mechanisms of papaverine.

## MATERIALS AND METHODS

### Drugs, solutions and chemicals

The following medications and chemicals were used in the experiments: acetylcholine (Dispersa Baeschlin, Germany), nifedipine, KT5720 and sucrose (Sigma Chemical Company, St. Louis, MO, USA).

The Krebs solution used (pH = 7.4) had the following content (mmol/l): NaCl – 120; KCl – 5.9;  $\text{CaCl}_2$  – 2.5;  $\text{MgCl}_2$  – 1.2;  $\text{NaH}_2\text{PO}_4$  – 1.2; NaHCO – 15.4, and glucose – 11.5. The pH of the solution was measured prior to each experiment by Microcomputer pH-meter 6201 (Jenco Electronics, UK). The substances used in preparing the Krebs

solution were produced by Merck (Darmstadt, Germany). The Krebs solution, as well as the sucrose and KCl solutions used in the single sucrose-gap method were tempered up to 37°C and constantly aerated with a gaseous mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ .

### Laboratory animals and SM preparations

The experiments involved 19 male Wistar rats, weighing 230-250 g, from whose stomachs we isolated SM. Two or three muscle strips were taken from one rat stomach.

The rats were provided by the Animal house of Medical University – Plovdiv, Bulgaria. The rats were housed in standard laboratory conditions (23-25°C, 50-55% humidity and 12/12h light/dark cycle) and fed with standard commercial food and given water ad libitum.

Decapitation was performed under ether-induced narcosis. The SM preparations were excised from the gastric corpus, separating the muscle tissue and preserving the mucosa intact. The preparations were obtained under conditions of continuous irrigation of the tissues with pre-aerated preparation solution containing NaCl/KCl/ $\text{CaCl}_2$  in the ratio of 27.2 to 1.1 to 1.

SM preparations of circular dissection, 12-13 mm in length and 1.0-1.1 mm in width, were used to record isometrically the contractile activity (CA); the preparations used to record bioelectric activity (BEA) by means of the single sucrose-gap technique were 26-28 mm long and 0.8-0.9 mm wide. All procedures were approved by the Institutional Animal Care Bulgaria and complied with the EU Directive 2010/63/EU. All efforts were made to minimize the number of animals used and their suffering.

### Synthesis of the desired compound

The synthesis of IQP is based on the initially formed aziridine ring. The ring is formed from naturally occurring amino acids through their reduction to  $\beta$ -amino alcohols.  $\beta$ -amino alcohols in turn proceed cyclization to produce the aziridine ring. The so produced aziridine ring is of interest for theoretical, bioorganic and medical chemistry. It is used as a substrate or synthetic intermediate for synthesis of a great number of molecules with biological activity. The synthesis of isoquinolines, molecules with significant pharmacologic applications is amongst them.

To achieve the goal, 10 mmol of an amino acid solution was coming to the boil in tetrahydrofuran with lithium-aluminum hydride added to the solution. The so received  $\beta$ -amino alcohol was allowed to interact with potassium hydroxide and tosyl chloride in water/dichloroethane solution. 2-methyl-1-tosylaziridine is the product of the reaction.

The second stage of the synthesis of our desired molecule was linked to the aziridine ring opening to produce 2-phenylethylamine, which in turn gives rise to isoquinoline derivatives. We used the principles of Gilbert's reaction<sup>13,14</sup> to make the ring opened. The reaction was perfor-

med as following: 4-bromo-1,2-dimethoxybromobenzen was added to a medium with tetrahydrofuran anhydrous and magnesium grit, and afterwards 2-methyl-1-tosylaziridine was added to the reagents. The reaction produces 1(3,4-dimethoxyphenyl)-propane-2-amine, which in turn was solved in dichloromethane. Once solved it was allowed to undergo acylation with 2-chloro-2-phenylacetyl chloride (to produce IQP) in the presence of trimethylamine. The target product is produced in high yields (95%) and purity after development and optimization of the method.

## Study design

In accordance with the structural resemblance of IQP and papaverine, and the hypothetical spasmolytic effect of the molecule, we considered that smooth muscles isolated from stomach would be a beneficial subject for our experiments. We examined the influence of IQP over the muscle tonus, bioelectrogenesis and the CA. Our experiment was focused on mechanisms which are specific for the action of papaverine.

The aim of the experiments with nifedipine was to demonstrate the rate of influence of IQP over the membrane, potential-dependent  $\text{Ca}^{2+}$  channels. The usage of KT5720, an inhibitor of the enzyme PKA, would clarify how much the activity of PKA and the intracellular levels of the cAMP could be affected by IQP.

## Method of studying BEA of isolated smooth muscles preparations (SMPs)

BEA was measured by the single sucrose-gap method. The muscle strips were divided into 3 zones: two electrode sections were flushed with isotonic KCl solution and Krebs solution (pH 7.4) respectively, and separated by a sucrose layer of high electric resistance. Krebs, KCl and sucrose bathing solutions ( $37^{\circ}\text{C}$ ) were continuously aerated with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Creation of a potential difference between the two end sections of the gap (control conditions) was achieved by means of silver non-polarizable, freshly chlorinated electrodes and after a 40-minute period of adaptation and stabilization was accepted as the initial (autocontrol).

CA were recorded from the fragment of the SMPs located in the section with Krebs solution. The membrane potential value was visualized by means of a Linseis recorder (Selb, Germany).

## Method of studying spontaneous CA of isolated SM preparations

The mechanical activity was isometrically recorded. The SM strips were placed in a tissue bath containing Krebs solution (20 ml) and were attached to a stationary glass holder at one end and to Swema tensodetectors (Stockholm, Sweden) at the other. The signal of the tensodetectors presenting the SM tissue contractile activity was amplified by

K. Tesar – D 486 amplifier (Germany) or Microtechna amplifier (Prague, Czech Republic). The mechanical activity was recorded on paper by Linseis polygraph recorder (Selb, Germany).

The value of the initial mechanical tension of the preparations, obtained by stretching the tension system corresponded to a tension force 10 mN. For the purposes of stabilizing the muscle tonus and spontaneous CA, about 60 minutes were allowed to elapse, during which period the Krebs solution in the tissue bath was changed 2-3 times. The baseline tonus value following this adaptation period was accepted as the zero one. The drug-induced alterations (contraction or relaxation), were recorded as a positive or negative change with regard to this value.

The influence of the substances under study (dissolved in double distilled water) was investigated after adding a precisely determined volume of concentrated solution of the respective substance, so that its required concentration in the tissue bath could be achieved. SM tissue vitality was tested by adding  $1 \times 10^{-6}$  mol/l acetylcholine at baseline twice and at the end of each application of the substances used.

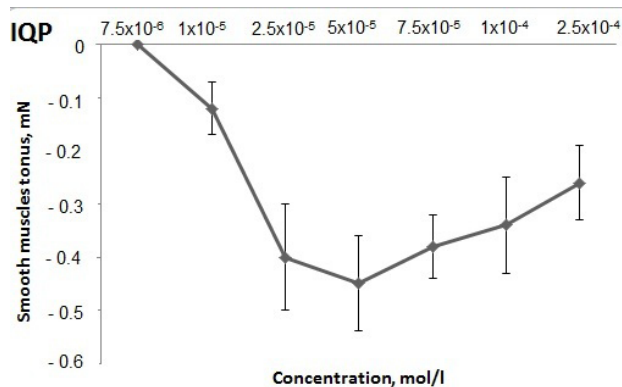
## Statistical analysis

The results obtained were statistically analysed using specialized software (STATISTICA 13.3, StatSoft 2017). All data were expressed as mean values  $\pm$  Standard Error of the mean; the number of muscle preparations used for each data point was indicated by n. The significance in comparing was determined by Student's t-test at confidence level 95% ( $p < 0.05$ ).

## RESULTS

### Effect of IQP on the contractile activity

SMPs do not respond to the presence of IQP, when treated with doses from  $7.5 \times 10^{-6}$  mol/l or lower. Concentrations higher than  $7.5 \times 10^{-5}$  mol/l provoke relaxation of the SM



**Figure 1.** Concentration-effect dependence of the effect of IQP on the muscle tonus of SM from stomach ( $n = 7$  - "n" indicates the number of rat circular gastric smooth muscle strips). The symbol minus "-" indicates the SM relaxation process.

illustrates the curve concentration/effect of the tonic SM reactions, obtained when SM were treated with IQP in a range  $7.5 \times 10^{-6} - 2.5 \times 10^{-4}$  mol/l (Fig. 1).

IQP also reduces the strength of the spontaneous phasic contractions. Their amplitude ( $1.08 \pm 0.03$  mN) decreases to  $0.19 \pm 0.02$  mN ( $N = 43$ ;  $p = 0.0032$ ) in 7-8 minutes after administration of  $5 \times 10^{-5}$  mol/l IQP ( $N$  is the number of consecutive spontaneous phasic contractions in 10 min). Isometric record of the IQP provoked reaction on the muscle tonus is present in Fig. 2.

### Effect of IQP on the spontaneous bioelectric activity

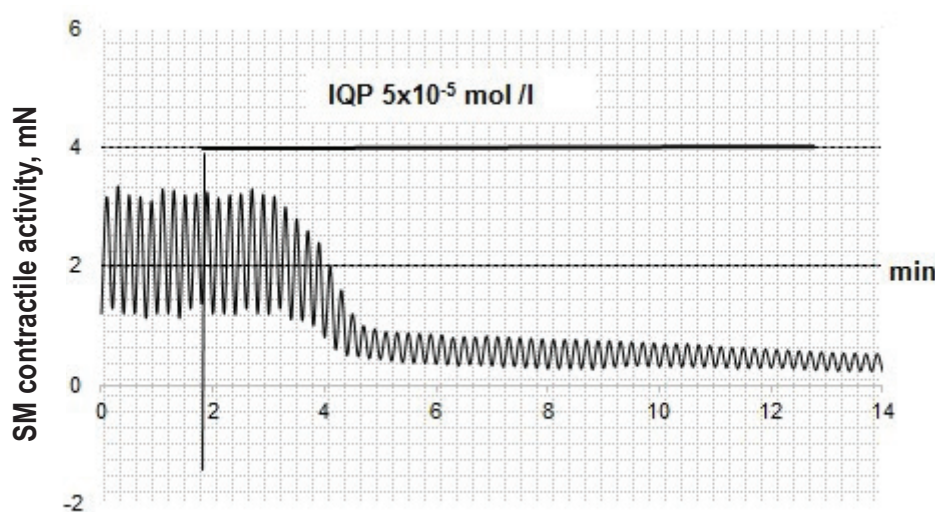
IQP at a concentration of  $5 \times 10^{-5}$  mol/l affects some indices

of the spontaneous bioelectrical activity of SM preparations. It was registered a tendency for reduction of the amplitude of the slow waves and statistically significant decrease of the frequency of the spike-potentials in 10 minutes after administration of IQP (Fig. 3 and Table 1).

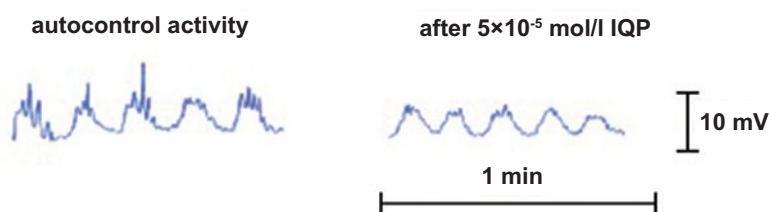
### Effects of nifedipine on the IQP-induced muscle relaxation

Nifedipine premedicated ( $1 \times 10^{-7}$  and  $5 \times 10^{-7}$  mol/l), gastric smooth muscle preparations showed lowering of the IQP-stimulated relaxation reaction. The rate of this reduction is proportional to the concentration of the muscle relaxant used during the experiment.

Nifedipine at concentrations of  $1 \times 10^{-7}$  mol/l and  $5 \times 10^{-7}$



**Figure 2.** Isometric record of the mechanical reactions of gastric smooth muscle elicited by the IQP, applied at concentration  $5 \times 10^{-5}$  mol/l.



**Figure 3.** Elements of an experimental record made by means of the single sucrose gap method of a bioelectric activity of SM preparation from the stomach of rat under control conditions (autocontrol) and 10 min after application of  $5 \times 10^{-5}$  mol/l IQP.

**Table 1.** Effects of IQP on bioelectric activity of rat circular gastric smooth muscles ( $n=6$ ). The comparison is between the initial values of the autocontrols and the changes in 10 minutes after being influenced by  $5 \times 10^{-5}$  mol/l IQP, \* -  $p < 0.05$ .

Bioelectric activity indicators		In the absence of IQP (autocontrol)	In the presence of $5 \times 10^{-5}$ mol/l IQP	<i>p</i>
Slow waves	number of waves, $\text{min}^{-1}$	$5.63 \pm 0.13$	$5.47 \pm 0.15$	0.07
	amplitude, mV	$5.25 \pm 0.17$	$5.10 \pm 0.11$	0.09
Spike potentials	number of potentials, $\text{min}^{-1}$	$20.57 \pm 1.68$	$6.04 \pm 0.12^*$	0.0001
	amplitude, mV	$2.75 \pm 0.89$	$0.12 \pm 0.08^*$	0.0001

**Table 2.** Reciprocal influence on the relaxing effectivity caused by nifedipine and IQP on rat circular gastric smooth muscles.

Impact agent	Autocontrol (mN)	Tonus of SMPs (mN) in the presence of	n	p
		<b>1×10<sup>-7</sup> mol/l nifedipine</b>		
5×10 <sup>-5</sup> mol/l IQP	-0.82±0.28	-0.45±0.29 *	9	0.014
		<b>5×10<sup>-7</sup> mol/l nifedipine</b>		
5×10 <sup>-5</sup> mol/l IQP	-0.79±0.19	-0.39±0.17 *	9	0.011
		<b>IQP 5×10<sup>-5</sup> mol/l</b>		
1×10 <sup>-7</sup> mol/l nifedipine	-0.41±0.18	0*	6	0.003
5×10 <sup>-7</sup> mol/l nifedipine	-0.39±0.11	0*	6	0.002

\*  $p < 0.05$ ; n: number of muscle preparations used for each data point. The comparison is between the autocontrol and remnant relaxing activity of each of the agents.

mol/l, accomplished after IQP at concentration 5×10<sup>-5</sup> mol/l, does not provoke SM response (Table 2).

### Effect of KT 5720 on the IQP-induced muscle relaxation

The inhibitor of PKA (5×10<sup>-6</sup> mol/l KT5720), significantly decreases the IQP-induced muscle relaxation from -1.49±0.44 mN to -0.58±0.19 mN (n = 8;  $p=0.003$ ).

## DISCUSSION

IQP causes relaxation of gastric SM of rats. Its influence on the tonus and spontaneous CA is complex. The relaxation of SM appears and exists between very narrow concentration limits (1×10<sup>-5</sup> – 5×10<sup>-5</sup> mol/l). At higher concentrations (7.5×10<sup>-5</sup> – 2.5×10<sup>-4</sup> mol/l) the effect on muscle relaxation still lasts, although its strength is reduced. This reduction could be a result from the activity of an additional IQP-induced pathway that affects the SM contractile activity, restraining the effect of the initial relaxation activator.

We assume that SM relaxation is provoked by the effect of IQP on the Ca<sup>2+</sup> influx towards SM, an effect that leads to decreased intracellular Ca<sup>2+</sup> levels. This assumption is made on the basis of the results we got in single sucrose-gap experiments. It was found that IQP affects some parameters of the bioelectric activity of SM obtained from rat stomach (spike potentials). IQP reduces the amplitude of the slow waves, but has no effect on their frequency. The absence of IQP effects on the frequency provides indirect information<sup>15</sup> which indicates that the compound has relation neither to the process of bioelectrogenesis of pacemaker potentials from ICC-cells, nor to their spread in an ICC network.

IQP reduces both the frequency and amplitude of the spike-potentials. This indicates that IQP has the capability of restraining the Ca<sup>2+</sup> influx towards SM cells.<sup>16</sup> The manifestation of this ability over the mechanical activity can be signified by minimizing the strength of the spontaneous

phasic contractions. Calcium, which is needed for such an activity, enters the cells through membrane-bound potential-dependent Ca<sup>2+</sup> channels (mostly from L-type). Our results suggest that IQP possesses an inhibitory effect on these channels.

Nifedipine is a blocker of the L-type Ca<sup>2+</sup> channels.<sup>17,18</sup> The effect of IQP at concentrations we used it in our experiments reduces the relaxing effect of 1×10<sup>-7</sup> mol/l nifedipine. This confirms our hypothesis about the negative effect of IQP on the activity of these channels. At the same time, 1×10<sup>-7</sup> mol/l or even 5×10<sup>-7</sup> mol/l nifedipine does not decrease the relaxing effect of IQP. The presence of an IQP-induced relaxing effect on the background of nifedipine might be a sign for the existence of a different pathway, parallel to or alternative, through which the SM contractile activity can be affected.

The ability of IQP to cause relaxation of gastric smooth muscles was examined, as well. We assumed that such ability would exist, and it would be based on the structural similarities between IQP and papaverine. A proper tool to examine it is KT5720. KT5720 regulates many cAMP-dependent signal transduction cascades and intracellular reactions.<sup>19</sup> It is well known that many cAMP-dependent cascades require activation of PKA via its activator cAMP. The activation of the PKA is followed by phosphorylation of specific target structures in the cells.<sup>20,21</sup> KT5720 is capable of passing through the cell plasma membrane. It is a potent and highly specific inhibitor of PKA.<sup>22</sup> KT5720 blocks the PKA signalling pathways acting as a competitive inhibitor for the enzyme.<sup>23</sup> At the same time, it does not affect the enzyme activity of other kinases (PKC, PKG or MLCK) involved in the cell signalling and affecting the SM contractions.<sup>24</sup> Our results indicate that the relaxing effect of IQP is significantly reduced when the PKA is preliminary inhibited with 5×10<sup>-6</sup> mol/l KT5720. This gives us the reason to suggest that the effect of IQP on gastric SM results from stimulation of an intracellular, cAMP-dependent pathway.

The activation of PKA might contribute to the ability of IQP as a blocker of the Ca<sup>2+</sup> channels, hence both the processes that provoke IQP-dependent relaxation might be

synergistic. The effect also might be a result from PKA-dependent phosphorylation of the channels, and a reason for their inactivation in SM<sup>25</sup>, decreasing the Ca<sup>2+</sup> influx and SM relaxation, which were registered in our experiment.

The established effect of the newly synthesized molecule on bioelectrogenesis and contractile activity of isolated smooth muscle characterizes IQP as a substance with functional characteristics of spasmolytic with impact on gastric smooth muscle.

## Acknowledgements

This study is part of Scientific Project DPDP – 11/2018 of MU Plovdiv and we acknowledge the financial support.

## REFERENCES

1. Wong EH, Kemp JA, Priestley T, et al. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc Natl Acad Sci USA* 1986; 83(18):7104–8.
2. Ohkubo M, Kuno A, Katsuta K, et al. Studies on cerebral protective agents. IX. Synthesis of novel 1,2,3,4-tetrahydroisoquinolines as N-methyl-D-aspartate antagonists. *Chem Pharm Bull* 1996; 44(1):95–102.
3. Kubota H, Watanabe T, Kakefuda A, et al. Synthesis and pharmacological evaluation of N-acyl-1,2,3,4-tetrahydroisoquinoline derivatives as novel specific bradycardic agents. *Bioorg Med Chem* 2004; 12(5):871–82.
4. Ivanov I, Nikolova S, Aladjov D, et al. Synthesis and contractile activity of substituted 1,2,3,4-tetrahydroisoquinolines. *molecules* 2011; 16(8):7019–42.
5. Iguchi M, Nakajima T, Hisada T, et al. On the mechanism of papaverine inhibition of the voltage-dependent Ca<sup>++</sup> current in isolated smooth muscle cells from the guinea pig trachea. *J Pharmacol Exp Ther* 1992; 263(1):194–200.
6. Kukovetz WR, Pöc GH. Inhibition of cyclic-3',5'-nucleotide-phosphodiesterase as a possible mode of action papaverine and similarly acting drugs. *Naunyn Schmiedebergs Arch Pharmacol* 1970; 267(2):189–94.
7. Karaki H, Weiss A. Calcium channels in smooth muscle. *Gastroenterology* 1984; 87(4):960–70.
8. Becker S, Truss AJ, Jonas MC. The effect of the specific phosphodiesterase (PDE) inhibitors on human and rabbit cavernous tissue in vitro and in vivo. *J Urol* 1998; 159:1390–3.
9. Liu JK, Couldwell WT. Intra-arterial papaverine infusions for the treatment of cerebral vasospasm induced by aneurysmal subarachnoid hemorrhage. *Neurocritical Care* 2005; 2(2):124–32.
10. Poser CM. Letter: Papaverine in prophylactic treatment of migraine. *Lancet* 1974; 1(7869):1290.
11. Bella AJ, Brock GB. Intracavernous pharmacotherapy for erectile dysfunction. *Endocrine* 2004; 23(2-3):149–55.
12. Desvaux P. Intracavernous injections of prostaglandin E1 for erectile dysfunction: patient satisfaction and quality of sex life on long-term treatment. *J Sexual Med* 2007; 4(2):426–31.
13. Shirley DA. The synthesis of ketones from acid halides and organometallic compounds of magnesium, zinc, and cadmium. *Org React* 1954; 8:28–58.
14. Huryn DM. Carbanions of alkali and alkaline earth cations: (II) selectivity of carbonyl addition reactions. In: Trost BM, Fleming I, editors. *Comprehensive Organic Synthesis*, 2<sup>nd</sup> edition, Volume 1: Additions to C–X  $\pi$ -Bonds, Part 1. Elsevier Science: 1991; 49–75.
15. Van Helden DF, Laver DR, Holdsworth J, et al. Generation and propagation of gastric slow waves. *Clin Exp Pharmacol Physiol* 2010; 37(4):516–24.
16. Mangel AW, Evans ED. Depolarization-stimulated contractility of gastrointestinal smooth muscle in calcium-free solution: a review. *ISRN Gastroenterol* 2011; 2011:692528.
17. Nayler WG, Poole-Wilson P. Calcium antagonists: definition and mode of action *Basic Res Cardiol* 1981; 76:1-15.
18. Papatsonis PN, Lok CA, Bos JM, et al. Calcium channel blockers in the management of preterm labour and hypertension in pregnancy. *Eur J Obstet Gynecol* 2001; 97(2):122–40.
19. Murray AJ. Pharmacological PKA inhibition: all may not be what it seems. *Sci Signal* 2008; 1(22):re4.
20. MacDonald JA, Walker LA, Nakamoto RK, et al. Phosphorylation of telokin by cyclic nucleotide kinases and the identification of in vivo phosphorylation sites in smooth muscle. *FEBS Lett* 2000; 479(3):83–8.
21. Murthy KS, Zhou H, Grider JR, et al. Inhibition of sustained smooth muscle contraction by PKA and PKG preferentially mediated by phosphorylation of RhoA. *Am J Physiology-Gastrointestinal and Liver Physiol* 2003; 284(6):1006–16.
22. Cheng Q, Zhou Y. Novel role of KT5720 on regulating hyperpolarization-activated cyclic nucleotide-gated channel activity and dorsal root ganglion neuron excitability. *DNA Cell Biol* 2013; 32(6): 320-8.
23. Lin HH, Chang SJ, Shie HJ, et al. Ethanol inhibition of NMDA-induced responses and acute tolerance to the inhibition in rat rostral ventrolateral medulla in vivo: Involvement of cAMP-dependent protein kinases. *Neuropharmacology* 2006; 51(4):747–55.
24. Davies SP, Reddy H, Caivano M, et al. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *2000 Biochem J* 2000; 351(1):95–105.
25. Xiong Z, Sperelakis N. Regulation of L-type calcium channels of vascular smooth muscle cells. *J Mol Cell Cardiol* 1995; 27(1):75–91.
26. <https://statistica.software.informer.com/13.3/>

# Влияние вновь синтезированной молекулы (2-хлор-N-(1-(3,4-диметоксифенил)пропан-2-ил)-2-фенилацетамида) на биоэлектrogenез и сократительную активность изолированных гладких мышц

Вера Н. Гледачева<sup>1</sup>, Илияна Д. Стефанова<sup>1</sup>, Валери И. Славчев<sup>1</sup>, Райна Г. Ардашева<sup>1</sup>, Атанас Д. Крастев<sup>1</sup>, Стоянка А. Николова<sup>2</sup>, Кремена Е. Сарачева<sup>3</sup>, Даринка С. Димитрова<sup>4</sup>

<sup>1</sup> Кафедра медицинской физики и биофизики, Факультет фармации, Медицинский университет – Пловдив, Пловдив, Болгария

<sup>2</sup> Кафедра органической химии, Химический факультет, Пловдивский университет „Паисий Хилендарски“, Пловдив, Болгария

<sup>3</sup> Кафедра фармакологии и лекарственной токсикологии, Факультет фармации, Медицинский университет – Пловдив, Пловдив, Болгария

<sup>4</sup> Кафедра фармакологии и клинической фармакологии, Факультет медицины, Медицинский университет – Пловдив, Пловдив, Болгария

**Адрес для корреспонденции:** Вера Н. Гледачева, Кафедра медицинской физики и биофизики, Факультет фармации, Медицинский университет – Пловдив, бул. „Васил Априлов“ № 15А, 4002 Пловдив, Болгария; E-mail: vera\_m@abv.bg; Тел.: +359 883 481 486

**Дата получения:** 31 октября 2019 ♦ **Дата приемки:** 25 февраля 2020 ♦ **Дата публикации:** 30 сентября 2020

**Образец цитирования:** Gledacheva VN, Stefanova ID, Slavchev VI, Ardasheva RG, Kristev AD, Nikolova SA, Saracheva KE, Dimitrova DS. Impact of a newly synthesized molecule (2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide) on the bioelectrogenesis and the contractile activity of isolated smooth muscles. *Folia Med (Plovdiv)* 2020;62(3):532-8. doi: 10.3897/folmed.62.e47844.

## Резюме

**Введение:** Исследование потенциальных возможностей 2-хлор-N-(1-(3,4-диметоксифенил)пропан-2-ил)-2-фенилацетамида (IQR) для воздействия на биоэлектrogenез и сократительную активность изолированных гладких мышц (ГМ) желудка.

**Цель:** Учитывая структурное сходство между молекулами папаверина и IQR, целью настоящего исследования было изучение тех характеристик вновь синтезированной молекулы, которые потенциально могут влиять на мышечный тонус, спонтанную биоэлектrogenную и сократительную активность гладких мышц, изолированных из желудка на основе специфических механизмов папаверина.

**Материалы и методы:** Синтез IQR основан на изначально образованном азиридиновом кольце в соответствии с принципами реакции Гилберта. Влияние IQR на биоэлектrogenез и сократительную активность изолированных гладких мышц самцов крыс линии Wistar измеряли методом одиночного сахарозного мостика и регистрировали изометрически.

**Результаты:** IQR ( $1 \times 10^{-5}$  -  $2.5 \times 10^{-4}$  mol/l) вызывает расслабление мышц, вызывая изменения в двух процессах, которые влияют на механическую активность гладких мышц:

1. Блокированный приток  $Ca^{2+}$  через потенциально зависимые мембранные каналы  $Ca^{2+}$  с последующим снижением внутриклеточных уровней  $Ca^{2+}$ . С одной стороны, этот эффект подтверждается изменениями частоты и амплитуды пиковых потенциалов в экспериментах с сахарозным мостиком (sucrose-bridge experiments) при применении IQR. С другой стороны, эффект IQR снижался в присутствии нифедипина ( $1 \times 10^{-7}$  моль / л), блокатора  $Ca^{2+}$  каналов L-типа.

2. Активация АМР-зависимого сигнального каскада. Релаксирующий эффект IQR был значительно снижен в присутствии КТ5720 ( $5 \times 10^{-6}$  mol/l), ингибитора протеинкиназы А (ПКА). ПКА представляет собой фермент, активность которого связана со значительным количеством сАМР-опосредованных биологических эффектов.

**Заключение:** Мы предполагаем, что могут быть взаимосвязи между этими двумя IQR-зависимыми процессами, как ПКА-зависимое фосфорилирование каналов  $Ca^{2+}$  каналов L-типа. В гладких мышцах это вызывает реакцию инактивации.

## Ключевые слова

биомеханические процессы, желудочно-кишечные мышцы, изохинолиновый скелет, природные аминокислоты, новый синтез