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Activity of guanylyl cyclase activators on the reaction of tracheal smooth muscle contraction*

Działania aktywatorów cyklazy guanylanowej na reakcję skurczu mięśniówki gładkiej tchawicy

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Summary

Introduction: The subject of the study compare the influences of YC-1 guanylyl cyclase *activator* with ODQ guanylyl cyclase inhibitor on the tracheal smooth muscle contraction induced by carbachol. The study specified the influence of increasing concentrations of soluble guanylyl cyclase activators YC-1 and 8Br cGMP on the reaction of tracheal smooth muscle contraction released by carbachol. The author also examined the effect of increasing concentrations of soluble guanylyl cyclase inhibitor ODQ on the concentration-effect curves for carbachol.

Material/Methods: Testing was conducted on an isolated trachea of both sexes of Wistar rats with weight ranging between 350 g and 450 g. Tracheas were prepared in accordance with the Akcasu (1959) method in Szadujkis-Szadurski (1996) modification. Concentration-effect curves were determined with the use of cumulated concentration method, in accordance with the van Rossum method (1963) in Kenakin (2006) modification.

Results: According to conducted testing, activation of soluble guanylyl cyclase with the use of YC-1 and 8Br cGMP caused reduced reaction of the tracheal smooth muscle with carbachol on average to 80%. Comparing concentration-effect curves for carbachol before and after the use of 8Br cGMP, similar results were obtained for those released by YC-1.

On the other hand, increasing concentrations of guanylyl cyclase inhibitor – ODQ cause shift of curves to the left, decrease of EC_{50} value and an increase of maximum reaction to carbachol.

Conclusions: Carbachol, depending on concentration, causes tracheal smooth muscle contraction. According to testing, we can confirm that activation of guanylyl cyclase leads to reduction of the reaction of tracheal smooth muscle to carbachol on average up to 80%

Key words: **guanylyl cyclase • smooth muscle • trachea • carbachol • 8Br cGMP • ODQ • YC-1**

Streszczenie

Wstęp: Przedmiotem pracy jest określenie działania aktywatorów cyklazy guanylanowej na reakcję skurczu mięśniówki gładkiej tchawicy. W pracy oznaczano wpływ wzrastających stężeń aktywatora cyklazy guanylanowej YC-1 oraz 8Br cGMP na reakcję skurczu mięśniówki gładkiej wyzwalaną karbacholem. Badano także wpływ wzrastających stężeń inhibitora cyklazy guanylanowej ODQ na krzywe stężenie–efekt dla karbacholu.

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Materiał/Metody: Badania przeprowadzono na izolowanej tchawicy szczurów szczepu Wistar obu płci o masie 350–450 g. Tchawice preparowano zgodnie z metodą (Akcasu) (1959) w modyfikacji Szadujkis-Szadurski (1996). Krzywe stężenie–efekt wyznaczano metodą stężeń kumulowanych, zgodnie z metodą van Rossuma (1963) w modyfikacji Kenakin (2006).

Wyniki: Z przeprowadzonych badań wynika, że aktywacja cyklazy guanylanowej za pomocą YC-1 i 8Br cGMP powoduje obniżenie reakcji mięśniówki gładkiej tchawicy na karbacholu średnio do 80%. Z porównania krzywych stężenie–efekt dla karbacholu przed i po zastosowaniu 8Br cGMP uzyskano podobne wyniki do wyzwalanych przez YC-1.

Natomiast wzrastające stężenia inhibitora cyklazy guanylanowej – ODQ powodują przesunięcie krzywych w lewo, obniżenie wartości EC_{50} i podwyższenie maksymalnej reakcji na karbacholu. Można więc stwierdzić, że działanie ODQ jest odwrotne do działania 8Br cGMP i YC-1.

Wnioski: Karbachol w sposób zależny od stężenia powoduje skurcz mięśniówki gładkiej tchawicy. Z przeprowadzonych badań wynika, że aktywacja cyklazy guanylanowej powoduje obniżenie reakcji mięśniówki gładkiej tchawicy na karbachol średnio do 80%.

Słowa kluczowe: **cyklaza guanylanowa • mięśniówka gładka • tchawica • karbachol • 8Br cGMP • ODQ • YC-1**

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Introduction

Nitric oxide (NO) induces airway smooth muscle cell (SMC) relaxation, but the underlying mechanism is not well understood. In the airways and lungs, nitric oxide (NO) is produced by epithelial ciliated cells, type II alveolar cells, and neural fibers that innervate the airway smooth muscle cells (SMCs) [19]. The NO released by these cells decreases airway resistance and that NO, released by neural fibers, is a major nonadrenergic, noncholinergic neurotransmitter responsible for airway SMC relaxation [5]. Furthermore, the signaling cascade by which NO induces smooth muscle relaxation has been mainly studied on vascular smooth muscle cells. In these blood vessels, NO is synthesized in the endothelial cells and diffuses to the adjacent SMCs, where it activates soluble guanylate cyclase (sGC) to synthesize cGMP. From these studies it is well known that cGMP acts as a second messenger to activate cGMP-dependent PKG and/or other effector proteins, including ion channels, ion pumps, and phosphodiesterases (PDEs) [6, 14]. Thus cGMP were involved in the phosphorylation of one or more target molecules by PKG and/or a direct activation/inhibition of ion channels by cGMP are believed to lead to smooth muscle relaxation. For these reason, we investigated the effects of ODQ an inhibitor and YC-1 an activator of soluble guanylyl cyclase (CG_s) on airway SMC contraction induced by carbachol and we extended this study to investigate and compare the effects of ODQ and YC-1 with the effect of 8Br cGMP on airway relaxation.

The subject of the study is determination of the activity of the nitric oxide (NO)-independent activators of soluble guanylyl cyclase activators YC-1 and selctive inhibitors ODQ in the modulation of the reaction of tracheal smooth muscle contraction. The study specified the influence of increasing concentrations of guanylyl cyclase activators YC-1 and 8Br cGMP on the reaction of tracheal smooth muscle contraction released by carbachol. The author also examined the effect of increasing concentrations of guanylyl cyclase inhibitor ODQ on the concentration--responses curves for carbachol.

Natural activators of guanylyl cyclase include nitric oxide and carbon monoxide. Both nitric oxide (NO) and carbon monoxide (CO) activate soluble guanylyl cyclase, binding with the heme group of this enzyme [10,22]. NO is produced through the conversion of l-arginine to citrulline by the enzyme NO synthase (NOS). In the lung, endothelial NOS (eNOS) is found in both vascular endothelium and airway epithelium [15]. CO shows significantly lower activity in this process than NO [24]. NO and cGMP have an important role in regulating pulmonary vascular tone and development. NO produced by nitric oxide synthase (NOS) in pulmonary endothelial cells diffuses into subjacent smooth muscle cells (SMC) where it stimulates soluble guanylyl cyclase (sGC) to increase cGMP production [3,18].

Although cGMP interacts with several proteins in SMC, cGMP regulates pulmonary vascular tone primarily by stimulating cGMP-dependent protein kinase I (PKGI). Cyclic GMP-activated PKGI phosphorylates several cytosolic protein targets that regulate intracellular Ca^{2+} levels, the calcium sensitivity of the contraction apparatus, and thin filament proteins and thereby cause vasodilatation [3,18].

Smooth muscle contraction may be regulated by Ca^{2+} through two pathways initiated by depolarization and agonist,

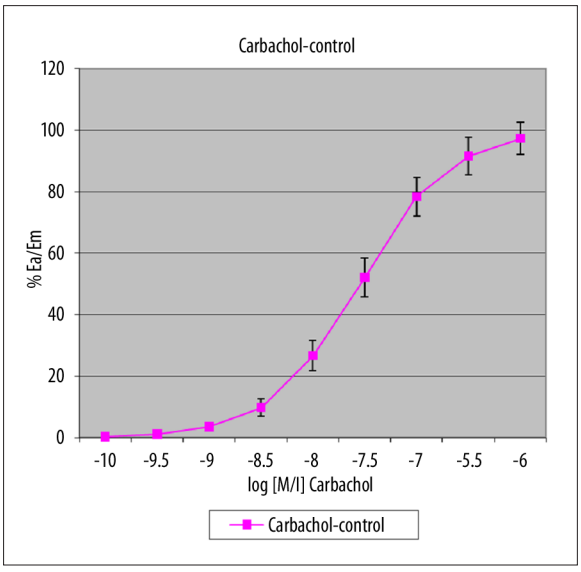

Fig. 1. Concentration-effect curves for carbachol 2.76 (±0.11)×10^{-8}. Points marked on the curve present average values and SE ± for n=9

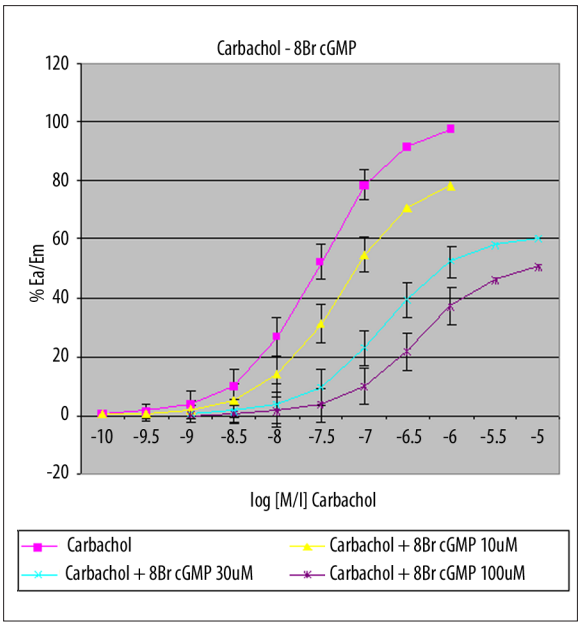

Fig. 2. Effect of increasing concentrations (from 10^{-8} to 10^{-7} [M/l]) 8Br cGMP on the concentration-effect curve for carbachol. Points marked on curves present average values and SE ± for n =9

respectively. Depolarization of the cell membrane activates voltage-gated Ca^{2+} channels resulting in Ca^{2+} influx, whereas agonist stimulation generally activates G protein-coupled receptors (GPCRs) leading to inositol 1,4,5-trisphosphate formation and Ca^{2+} release from the sarcoplasmic reticulum. The increase in cytosolic Ca^{2+} leads to smooth muscle contraction through myosin light chain kinase (MLCK) activation by Ca^{2+}/calmodulin and myosin regulatory light chain (RLC) phosphorylation. Additionally, activation of GPCRs leads to inactivation of MLCP by agonist-induced protein kinase C (PKC) and RhoA/ROCK activation. These inhibitory mechanisms thus enhance RLC phosphorylation and force development [23].

Tests, conducted in this study, indicate that in addition to the modulating component related to guanylyl cyclase in reaction of contraction to carbachol, there is a component independent of this nucleotide participating in this process.

Material and Methods

Testing was conducted on an isolated trachea of both sexes of Wistar rats with weight ranging between 350 g and 450 g. Tracheas were prepared in accordance with the Akcasu method (1959) [1] in Szadujkis-Szadurski modification (1996) [20]. A chain of combined segments of the trachea with 4 cm in length was placed in a dish for isolated organs. This dish was filled with Krebs fluid and oxidized, after previous addition of 5% CO_2. Reaction of the trachea was registered with the use of isotonic transducer Biograf F-60 and recorded by polyphysiograph Narco Bio-System Narcotrace 40 (USA). Tested compounds were added directly to the dish, in which the trachea was placed.

Concentration-effect curves for tested agonists were determined with the use of traditional van Rossum's pharmacometric method. EC_{50} values were determined using the linear regression method for the range between 20% and 80% of reaction. The value of dissociation constant of agonist-receptor complex was determined with the use of the Furchgott and Bursztyn method in Kenakin T modification (1997) [13].

The following reagents were used in testing: Carbachol, 8 Br cGMP (Beringher), 3-(5'-hydroxymethyl-2'-furyl)--1-benzylindazole (YC-1), (Sigma), 1,2,4-oxodiazolo-[4,3--a]quinoxalin-1-one (ODQ) (Sigma).

The experiments were carried out using of Krebs' fluid (normal) – PSS – composition: NaCl (71.8 mM/L), KCl (4.7 mM/L), $CaCl_2$ (1.7 mM/L), $NaHCO_3$ (28.4 mM/L), $MgSO_4$ (2.4 mM/L), KH_2PO_4 (1.2 mM/L), glucose (11.1 mM/L) with the addition of EGTA (30 µM/L).

Results

Carbachol in the range of concentrations between 10^{-10} and 10^{-6} M/l leads to tracheal smooth muscle contraction, dependent on concentration. The average EC_{50} value was determined from concentration-effect curves for carbachol, amounting to 2.76 (±0.11)×10^{-8} M/l for n=9.

Results are shown on Fig. 1.

Increasing concentration 8Br cGMP (10–100 µM/l) causes shifting of the concentration –response curve to the right with the decrease of maximum reaction to carbachol. Under the influence of this activity, EC_{50} for carbachol increases along with an increase of concentration 8Br cGMP. Average values EC_{50} for carbachol are presented in Table 1.

Increasing concentration YC-1(1–10 µM/l) causes shift of the curve into the right, with the decrease of maximum reaction to carbachol. Under the influence of the activity, EC_{50} for carbachol increases along with concentration YC-1. Average values EC_{50} for carbachol are presented in Table 2.

Table 1. Influence of carbachol on the reaction of tracheal smooth muscle contraction before and after the use of increasing concentrations 8Br cGMP

Drug	n	EC_{50} (± SE)
Carbachol	9	2.76 (± 0.11)×10^{-8}
Carbachol + 8Br cGMP 10 μM	9	4.92 (± 0.07)×10^{-8}
Carbachol+ 8Br cGMP 30 μM	9	1.67 (± 0.05)×10^{-7}
Carbachol+ 8Br cGMP 100 μM	9	4.31 (± 0.04)×10^{-7}

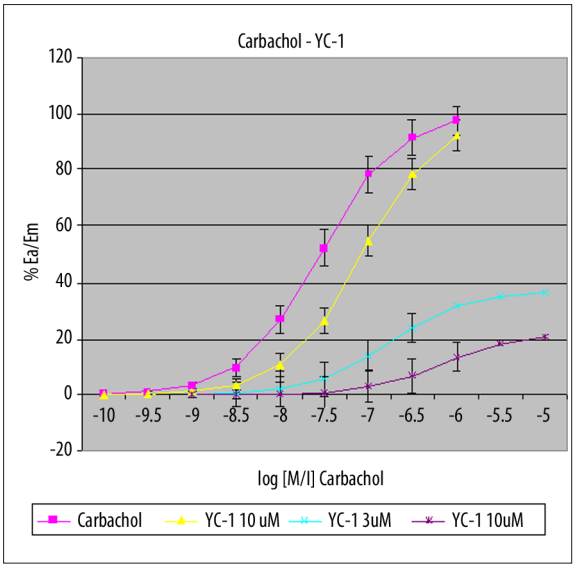

Fig. 3. Effect of increasing concentrations (from 10^{-8} to 10^{-7} [M/l]) YC-1 on the concentration-effect curve for carbachol. Points marked on curves present average values and SE ± for n =9

Increasing concentration ODQ (10–100 μM/l) causes shift of the curve into the left with simultaneous increase of maximum reaction to carbachol. Average value Em for carbachol under the influence of ODQ increases by 31(±6.6)%. Under the presence of ODQ, the average value EC_{50} for carbachol decreases from 2.76 (±0.11)×10^{-8} M/l to 4.11 (±0.14)×10^{-9} M/l. Average results obtained in this series of experiments are summarized in Table 3.

Discussion

In the airways and lungs, nitric oxide (NO) is produced by epithelial ciliated cells, type II alveolar cells, and neural fibers that innervate the airway smooth muscle cells [19]. Earlier research on modulating activity of cyclical nucleotides on the reaction of tracheal smooth muscle contraction indicated that cAMP and analogy of this nucleotide cause both relaxation and reduce the reaction of tracheal smooth muscle to contracting agents. Similar activity modulating smooth muscle contraction induces NO which activate of the soluble guanylyl cyclase and 8Br cGMP permeable analog of cGMP [4,8,11,12].

According to presented research, 8 Br cGMP – induced concentration depending reduces the reaction of tracheal smooth muscle contraction induced by carbachol. Under the influence of this cyclic nucleotide, concentration-effect

Table 2. Influence of carbachol on the reaction of tracheal smooth muscle contraction before and after the use of increasing concentrations YC-1

Drug	n	EC_{50} (±SE)
Carbachol	9	2.76 (±0.11)×10^{-8}
Carbachol + YC-1 1 μM	9	8.3 (±0.04)×10^{-8}
Carbachol+ YC-1 3 μM	9	1.7 (±0.07)×10^{-7}
Carbachol+ YC-1 10 μM	9	6.71 (±0.06)×10^{-7}

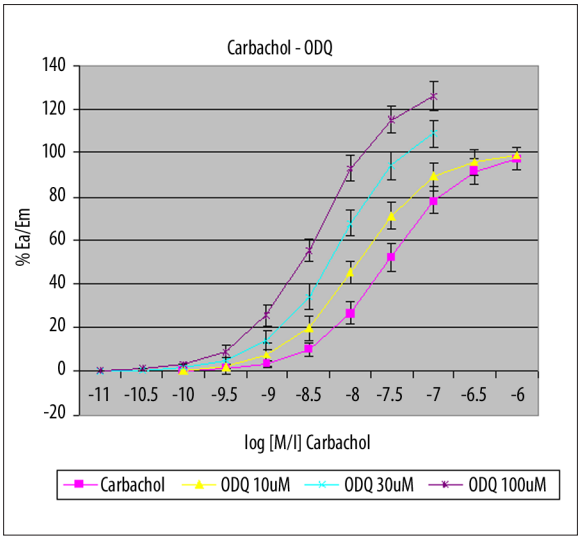

Fig. 4. Effect of increasing concentrations (from 10^{-9} to 10^{-8} [M/l]) ODQ on the concentration-effect curve for carbachol. Points marked on curves present average values and SE ± for n =9

Table 3. Influence of carbachol on the reaction of tracheal smooth muscle contraction before and after the use of increasing concentrations ODQ

Drug	n	EC_{50} (±SE)
Carbachol	9	2.76 (±0.11)×10^{-8}
Carbachol + ODQ 10 μM	9	1.21 (±0.23)×10^{-8}
Carbachol+ ODQ 30 μM	9	7.27 (±0.16)×10^{-9}
Carbachol+ ODQ 100 μM	9	4.11 (±0.14)×10^{-9}

curves for carbachol shift to the right with simultaneous reduction of the effect of maximum reaction. From the analysis of concentration-effect curves for carbachol, determined before and after the use of 8Br cGMP, we can deduce that this nucleotide behaves like allosteric antagonist. Further testing established that YC-1 selective activator of soluble guanylyl cyclase modulates in a similar way reaction of the trachea to carbachol. Antagonistic action of YC-1 in relation to carbachol in accordance with the principles of the receptor theory also meets the conditions of allosteric antagonist [2,7,13,21].

Additional testing analyzed the influence of soluble guanylyl cyclase inhibitor – ODQ on the reaction of tracheal smooth muscle contraction induced by carbachol. The

analysis of concentration responses curves (CRC) to carbachol shows that the increasing concentrations of ODQ have a statistically significant effect on the shift of curves to the left with simultaneous reduction of EC_{50} values and increase of maximum reaction to carbachol by 31(±6.6)% for n=9. The activity of ODQ is opposite to the activity of 8Br cGMP and YC-1 [9,11].

Increase of cGMP results in decrease of the tracheal smooth muscle contraction.

These results suggest a possibility of the use of activators of guanylyl cyclase in treatment of spastic bronchitis [8,16].

Conclusions

- Carbachol, depending on concentration, causes tracheal smooth muscle contraction.
- According to testing, we can confirm that activation of guanylate cyclase leads to reduction of the reaction of tracheal smooth muscle to carbachol on average up to 80%.
- Comparing concentration-effect curves for carbachol, before and after the use of 8Br cGMP, similar results were obtained for those released by YC-1.
- It indicates that regardless of modulating component related to guanylate cyclase in reaction of contraction to carbachol, a component independent of this nucleotide participates in the process.
- Confirmation of participation of CG and cGMP in regulation of tracheal smooth muscle contraction released by carbachol was obtained by the use of inhibitor ODQ.

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References

[1] Akcasu A.: The physiologic and pharmacologic characteristics of tracheal muscle. Arch. Int. Pharmacodyn. Ther., 1959; 122: 201–207

[2] Alexander S., Mathie A., Peters J.A.: Guide to receptors and channels, 2nd edition. Br. J. Pharmacol., 2007; 150: S1

[3] Bachiller P.R., Nakanishi H., Roberts J.D.Jr: Transforming growth factor-β modulates the expression of nitric oxide signaling enzymes in the injured developing lung and in vascular smooth muscle cells. Am. J. Physiol. Lung Cell Mol. Physiol., 2010; 298: L324–L334

[4] Baumert M.: Tlenek azotu – lekarz czy zabójca? Wiad. Lekarskie, 2005; 58: 7–8

[5] Belvisi M.G., Ward J.K., Mitchell J.A., Barnes P.J.: Nitric oxide as a neurotransmitter in human airways. Arch. Int. Pharmacodyn. Ther., 1995; 329: 97–110

[6] Carvajal J.A., Germain A.M., Huidobro-Toro J.P., Weiner C.P.: Molecular mechanism of cGMP-mediated smooth muscle relaxation. J. Cell. Physiol., 2000; 184: 409–420

[7] Condorelli P., George S.C.: In vivo control of soluble guanylate cyclase activation by nitric oxide: a kinetic analysis. Biophys. J., 2001; 80: 2110–2119

[8] Godber B.L., Doell J.J., Sapkota G.P., Blake D.R., Stevens C.R., Eisenthal R., Harrison R.: Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. J. Biol. Chem., 2000; 275: 7757–7763

[9] Gorczyca W.A.: Cyklazy guanylowe. Postępy Hig. Med. Dośw., 1999; 53: 209–222

[10] Gregory E.M., Goscin S.A., Fridovich I.: Superoxide dismutase and oxygen toxicity in a eukaryote. J. Bacteriology, 1974; 117: 456–460

[11] Jaffrey S.R., Snyder S.H.: Nitric oxide: a neural messenger. Ann. Rev. Cell Dev. Biol., 1995; 11: 417–440

[12] Kellogg E.W. III, Fridovich I.: Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J. Biol. Chem., 1975; 250: 8812–8817

[13] Kenakin T.: Pharmacologic analysis of drug – receptor interaction. 3rd ed., Raven Press, New York, 1997

[14] Kitazawa T., Semba S., Huh Y.H., Kitazawa K., Eto M.: Nitric oxide-induced biphasic mechanism of vascular relaxation via dephosphorylation of CPI-17 and MYPT1. J. Physiol., 2009; 587: 3587–3603

[15] MacRitchie A.N., Albertine K.H., Sun J., Lei P.S., Jensen S.C., Freestone A.A., Clair P.M., Dahl M.J, Godfrey E.A., Carlton D.P., Bland R.D.: Reduced endothelial nitric oxide synthase in lungs of chronically ventilated preterm lambs. Am. J. Physiol. Lung Cell Mol. Physiol., 2001; 281: L1011–L1020

[16] Mizuno T., Watanabe M., Sakamoto T., Sunamori M.: L-arginine, a nitric oxide precursor, attenuates ischemia – reperfusion injury by inhibiting inositol-1,4,5-triphosphate, J. Thorac. Cardiovasc. Surg., 1998; 115: 931–936

[17] Perez-Zoghbi J.F., Bai Y., Sanderson M.J.: Nitric oxide induces airway smooth muscle cell relaxation by decreasing the frequency of agonist-induced Ca^{2+} oscillations. J. Gen. Physiol., 2010; 135: 247–259

[18] Pilz R.B., Broderick K.E.: Role of cyclic GMP in gene regulation. Front Biosci., 2005; 10: 1239–1268

[19] Ricciardolo F.L., Sterk P.J., Gaston B., Folkerts G.: Nitric oxide in health and disease of the respiratory system. Physiol. Rev., 2004; 84: 731–765

[20] Szadujkis-Szadurski L., Berti F.: Pharmacological efects of cyclic nucleotides and their derivatives on tracheal smooth muscle. Pharm. Res. Comm., 1972; 4: 63–69

[21] Toutouzas P.C., Tousoulis D., Davies G.J.: Nitric oxide synthesis in atherosclerosis. Eur. Heart J., 1998; 19: 1504–1511

[22] Wang R., Wang Z., Wu L., Hanna S.T., Peterson-Wakeman R.: Reduced vasorelaxant effect of carbon monoxide in diabetes and the underlying mechanisms. Diabetes, 50, 2001; 166–174

[23] Zhang W.C., Peng Y.J., Zhang G.S., He W.Q., Qiao Y.N., Dong Y.Y.: Myosin light chain kinase is necessary for tonic airway smooth. J. Biol. Chem., 2010; 285: 5522–5531

[24] Zweier J.L., Samouilov A., Kuppusamy P.: Non-enzymatic nitric oxide synthesis in biological systems. Biochim. Biophys. Acta, 1999; 1411: 250–262

The authors have no potential conflicts of interest to declare.