B-5019
[1-5]

RESEARCH ARTICLE Indian Journal of Animal Research

Sodium Nitroprusside Inhibits Detrusor Muscle Contraction through G-protein Mediated Inhibition of Intracellular Ca^{2+} Release

L.N. Navya[1], Dilip K. Deka[1], D.C. Roy[1], J. Sarma[1], A. Hazarika[1]

10.18805/IJAR.B-5019

ABSTRACT

Background: Nitric oxide (NO) physiology is very complex in detrusor smooth muscle (DSM). While NO relaxes detrusor in some species, in contrary, it produces contraction in other. NO donor sodium nitroprusside (SNP) was found to inhibit CCh-induced contraction in goat DSM in earlier studies, but it is not known how SNP produces inhibitory action on goat DSM. Therefore, the aim of this *in vitro* study was to investigate the mechanism of inhibitory action of SNP pertaining to involvement of G- protein and other second messenger on goat DSM.

Methods: Goat detrusor muscle strips collected from local abattoir were mounted in a thermostatically controlled (37°± 0.5°C) organ bath (20 ml capacity) containing physiological solution. Following 1 hr of equilibration, carbachol (CCh) (10^{-5} M) induced sub-maximal contraction was elicited both in the absence and presence of SNP (10^{-5} M). Involvement of NO and guanylyl cyclase was assessed using nitric oxide synthase inhibitor L-NAME (10^{-5} M) and guanylyl cyclase inhibitor ODQ (10^{-5} M). Experiments were also carried out in the presence of G-protein activator aluminium fluoride (AlF) (10^{-5} M), protein kinase C (PKC) activator phorbol-12-myristate (PMA) (10^{-5} M), nifedipine, low Ca^{2+} PSS, theophylline and Zero Ca^{2+} PSS.

Result: Inhibitory effect of SNP (10^{-5} M) on CCh-induced contraction on goat DSM was reversed by nitric oxide synthase inhibitor L-name (10^{-5} M) and guanylyl cyclase inhibitor ODQ (10^{-5} M). Prior incubation of the tissues with AlF and PMA also reversed the SNP mediated inhibition. On the other hand, nifedipine and low Ca^{2+} PSS; and theophylline and zero Ca^{2+} PSS potentiated the SNP-mediated inhibition. Thus, the present study shows that SNP elicited inhibitory effect on goat DSM is both NO-dependent and cGMP-dependent. In addition, the result shows that SNP inhibits G-protein coupled PKC leading to inhibition of both Ca^{2+} entry and intracellular Ca^{2+} release with a resultant decrease in intracellular Ca^{2+}.

Key words: Detrusor, Intracellular Ca^{2+}, PKC, SNP.

INTRODUCTION

Detrusor over activity (DO) is a major concern for both man and animals particularly dog. Detrusor contraction requires an increase in intracellular concentrations of Ca^{2+} similar to vascular and other types of smooth muscle. It was shown that removal of extracellular Ca^{2+} had impaired muscarinic receptor-mediated bladder contraction (Jegior *et al*., 2001). The relative role of influx of Ca^{2+} from the extracellular space and mobilization of Ca^{2+} from intracellular stores are species-dependent (Wuest *et al*., 2007).

The physiological effect of nitric oxide (NO) on detrusor is species specific. While, NO relaxes detrusor muscle of rat (Chung *et al.*, 1996), human (James *et al.*, 1993) and sheep (Thornbury *et al.*, 1992), it produces contraction of the detrusor in guinea pig (Moon, 2000) and mouse (Fujiwara *et al.*, 2000). A more complex response of contraction followed by relaxation was also observed in human detrusor strips in response to NO donor (Moon, 2002).

It is now known that the relaxant or inhibitory effects of NO are mediated through second messengers in different tissues. For example, in vascular smooth muscle, NO-cGMP-mediated relaxation caused increased sequestration of cystosolic Ca^{2+} by activation of Ca^{2+} - ATPase pump with resultant decrease in intracellular Ca^{2+} and attenuation of voltage-dependent Ca^{2+} channels (Griffith, 1994). Similarly, inactivation of the cGMP-dependent protein kinase gene (cGK1) abolished NO/cGMP-dependent relaxation of urethral smooth muscle in mice (Persson *et al.*, 2000). An increase cGMP to certain level by SNP was without relaxation while an increase cAMP level was linked to rabbit detrusor muscle relaxation (Qui *et al.*, 2002). Similarly, inhibition of detrusor contractility in goat by a PDE_5 inhibitor avanafil was linked to an increase in cGMP (Dhruva *et al.*, 2019).

Considering the fact that SNP produced inhibitory effect against CCh-induced contraction on goat DSM (Barua

[1]College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781 022, Assam, India.

Corresponding Author: Dilip K. Deka, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781 022, Assam, India. Email: drdilipdeka@rediffmail.com

How to cite this article: Navya, L.N., Deka, D.K., Roy, D.C., Sarma, J. and Hazarika, A. (2023). Sodium Nitroprusside Inhibits Detrusor Muscle Contraction through G-Protein Mediated Inhibition of Intracellular Ca^{2+} Release Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5019.

Submitted: 12-09-2022 **Accepted:** 06-02-2023 **Online:** 09-03-2023

et al., 2010), the present study was undertaken to investigate its mechanism of inhibitory action pertaining to involvement of G protein and PKC.

MATERIALS AND METHODS

Urinary bladders from freshly slaughtered goats were collected and transported to the laboratory within 20-30 min of slaughter in cold oxygenated physiological modified Krebs' solution [Composition mM: NaCl 120, KCl 5.9 $MgCl_2$ 1.2, $CaCl_2.2H_2O$ 2.5, $NaHCO_3$ 15, NaH_2PO_4 1.2, Glucose (Dextrose) 11] from local abattoir in and around Khanapara, Guwahati (Assam), India. The experiments were conducted between the period of August, 2019 and Sept, 2020 in the Department of Pharmacology and Toxicology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam. The present study had the approval of the institutional animal ethics committee vide approval No. 770/Re/S/03/CPCSEA/AAU/IAEC/10-20/781 dated 23.12.2019.

The neck and dome parts were removed and and the bladders were cut open with the help of a scalpel in petri-dish containing aerated PSS. The fascia and urothelium were removed and DSM strips of about 2-3 mm breadth and 4-5mm length were dissected out. The strips were tied on either ends with thread, mounted into a thermostatically controlled (37°±0.5°C) organ bath (20 ml capacity) containing PSS and were continuously aerated. The tissue was allowed to equilibrate under a resting tension of 1 g for a period of 1 h and bathing fluid was changed ever 15 min during this period. CCh (10^{-5} M)-induced sub-maximal contraction was produced in absence and presence of SNP. As consistent with the earlier report, SNP produced inhibitory effect on CCh-induced contraction and different modulators of NO and other second messengers were used to see their influence on the SNP-evoked inhibition. Sufficient wash was given in between successive contraction. Isometric contractions were recorded by means of a force displacement transducer connected to a single channel physiograph (Medicaid, India).

Chemicals

All chemicals used for preparing the modified Krebs' solution were of analytical grade. The drug solutions meant for investigation of the experiment were either freshly prepared or taken from stock solutions stored at 4°C in the refrigerator. The stock solutions were replaced after 2 weeks. Sodium nitroprusside (SNP) (Narang chemical industry) was freshly prepared as 10^{-2} M solution whenever used. The following drug solutions were prepared in distilled water unless otherwise specified - Aluminium Fluoride (Loba chemie) 10^{-3} M, 8-Br-cGMP (Sigma) 10^{-2} M, Carbachol (Merck) 10^{-2} M, L-NAME (Sigma) 10^{-2} M, Nifedipine (Calbiochem) 10^{-3} M in DMSO, ODQ (Calbiochem) 10^{-2} M, Phorbol-12-Myristate, 13-acetate (PMA) (Acros Organics) 10^{-3} M in DMSO, Strontium Chloride (Fischer Scientific) 10^{-2} M, Theophylline (Loba chemie) 10^{-3} M in 0.1N HCl.

Statistical method

The results are presented as Mean ± Standard error of mean (n). Students''t' (unpaired) test was employed to measure the level of significance with $p<0.05$.

RESULTS AND DISCUSSION

Characteristics of CCh responses and effect of SNP

Sub-maximal contraction induced by CCh (10^{-5} M) on goat DSM came to a steady state after 10-15 minutes of application. Repeated application of CCh (10^{-5} M) did not alter the amplitude of contraction (data not shown). Incubation of the tissues with SNP (10^{-5} M) reduced the amplitudes of CCh-induced contraction. Thus, CCh-induced absolute force of contraction was 0.64±0.03 g (n=30, pooled data) in control as against 0.36±0.025 g (n = 22, pooled data) in SNP-treated tissues (Fig 1 and 2).

Effect of ODQ and L-NAME on SNP-effect

ODQ, an inhibitor of soluble guanylyl cyclase (sGC), was used to see the effect of sGC on SNP elicited inhibition. After obtaining CCh (10^{-5} M)-induced contraction in the absence and in presence of SNP (10^{-5} M), DSM strips were incubated with ODQ (10^{-5} M) for 5 min and then SNP for another 5 min. ODQ (10^{-5} M) reversed the SNP evoked inhibition. Thus, the CCh-elicited absolute force of contraction obtained in presence of ODQ plus SNP was 0.54±0.04 g (n = 4) as against 0.36±0.025 g (n = 22) in SNP-treated tissues (Fig 2). The reversal by ODQ of anti-contractile effect of SNP on CCh-induced contraction of goat DSM indicates that SNP produces inhibitory effect by activating guanylyl cyclase and thereby an increase in cGMP level in the goat DSM. An increase in cGMP was linked to the inhibitory effect of PDE inhibitor on detrusor contractility (Dhruva *et al.*, 2019).

L-NAME, an L-arginine analogue, was used to see the role of NO on the SNP evoked inhibition. CCh (10^{-5} M)-induced contraction was obtained before and after incubation with SNP (10^{-5} M) followed by L-NAME (10^{-5} M) plus SNP. Incubation of the tissues with L-NAME (10^{-5} M) reversed the anti-contractile effect of SNP (10^{-5} M). Thus, the absolute force of contractions obtained in L-NAME plus SNP was 0.53±0.03 g (n = 4) as against 0.36±0.025 g (n = 22) in SNP-treated tissues ($p<0.05$). We have observed that anti-contractile effect of SNP was reversed by L-arginine analogue L-name. As such, NO might be an inhibitory neurotransmitter in goat DSM like that of rat (Chung *et al.*, 1996), human (James *et al.*, 1993) and sheep (Thornbury *et al.*, 1992) and the effect of SNP might have been mediated through NO generation in this tissue. Both nNOS and eNOS were reported to be expressed in urothelial and detrusor cells (Satake *et al.*, 2017).

Effect of AlF and PMA on SNP-elicited anti-contractile effect

AlF is an activator of G-proteins and hence, was used to see the role of G-protein on anti-contractile effect of SNP on goat DSM. CCh (10^{-5} M)-induced contraction was

obtained in absence and in presence of AlF (10^{-5} M) with a contact period of 5 min. Thereafter, CCh-induced contraction was again obtained in the presence of SNP (10^{-5} M) and in the presence of AlF (10^{-5} M) plus SNP. DSM strips incubated with AlF (10^{-5} M) not only showed an increase in the CCh-induced absolute force of contraction, but also reversed the anti-contractile effect of SNP. Thus, the CCh-induced absolute force of contraction in presence of AlF was 1.0±0.12 g (n = 4) as against 0.64±0.03 g (n = 30, pooled data) in control ($p<0.05$). Similarly, the CCh-induced absolute force of contraction of the AlF plus SNP treated DSM tissues was 0.70±0.11 g (n = 4) as against 0.36±0.025 g (n = 22, pooled data) in SNP-treated tissues ($p<0.05$) (Fig 1 and 2).

CCh -induced M_3 receptor activated contraction was linked to G protein-coupled activation of PKC and Rho/ROCK (Wang *et al.*, 2017, Hypolite and Malykhina, 2015) apart from Ca^{2+}-influx through VDCC with activation of Rho kinase and minor activation of PLD and receptor-operated Ca^{2+} channels in UBSM (Schneider *et al.*, 2004). Therefore, the potentiating effect of AlF on the amplitude of CCh-induced contraction indicates the involvement of G-protein coupled PLD and PKC activation on goat DSM in the present study.

To substantiate the role of PKC in the SNP evoked inhibition, PMA, an activator of PKC was used. CCh-elicited contraction was obtained in absence and presence of SNP (10^{-5} M) and then in presence of PMA (10^{-5} M) plus SNP (10^{-5} M). As expected, PMA also reversed the anti-contractile effect of SNP similar to AlF (Fig 2). Thus, the absolute force of contraction induced by CCh (10^{-5} M) in presence of PMA plus SNP was 0.51±0.04 g (n = 4) as against 0.36±0.025 g (n=22) in SNP-treated tissues ($p<0.05$).

Effect of nifedipine and low Ca^{2+} - PSS on SNP-evoked inhibition

In order to investigate the role of extracellular Ca^{2+} on SNP evoked inhibitory effect, experiments were carried out using $Ca_V1.2$ channel blocker nifedipine and low Ca^{2+} PSS. For this, CCh (10^{-5} M)-induced contraction was obtained in absence and in presence of SNP (10^{-5} M) and then in presence of nifedipine (10^{-5} M) plus SNP (10^{-5} M). Incubation of the tissues with nifedipine potentiated the SNP evoked inhibition (Fig 3). Thus, the absolute force of contraction induced by CCh (10^{-5} M) in presence of nifedipine plus SNP was 0.22±0.009 g (n = 6) as against 0.36 ± 0.025 g (n = 22) in SNP-treated tissues ($p<0.05$). The potentiating effect in this study is attributed to involvement of two independent mechanisms-i) inhibition of receptor operated Ca^{2+} channels through G-protein coupled inhibition of PLD/PKC by SNP and ii) inhibition of $Ca_V1.2$ channel by nifedipine.

In the second set of experiments, CCh-elicited contraction was obtained in normal PSS and in low Ca^{2+} PSS ($CaCl_2$ was reduced to 0.63 M). Thereafter, UBSM strips were incubated with SNP (10^{-5} M) in low Ca^{2+} PSS followed again by CCh-induced contraction. Fig. 3 shows that CCh-induced contraction, which was reduced in low Ca^{2+} PSS, was further reduced in presence of SNP. Thus, the CCh-induced absolute force of contraction was 0.23±0.03 g (n = 4) in low Ca^{2+} PSS and 0.06±0.01 g (n = 4) in SNP plus low Ca^{2+} PSS ($p<0.05$). The augmented reduction of CCh-induced contraction by SNP in low Ca^{2+} PSS also justifies the inhibition of Ca^{2+}- influx by SNP. In vascular smooth muscle, SNP evoked inhibition was reported to be due to inhibition of Ca^{2+}-influx by voltage operated channel (Schmid *et al.*, 2018).

Effect of theophylline and Zero Ca^{2+}- PSS on SNP-mediated effect

Theophylline is a nonselective phosphodiesterse (PDE) inhibitor and was used in the present study to see the role

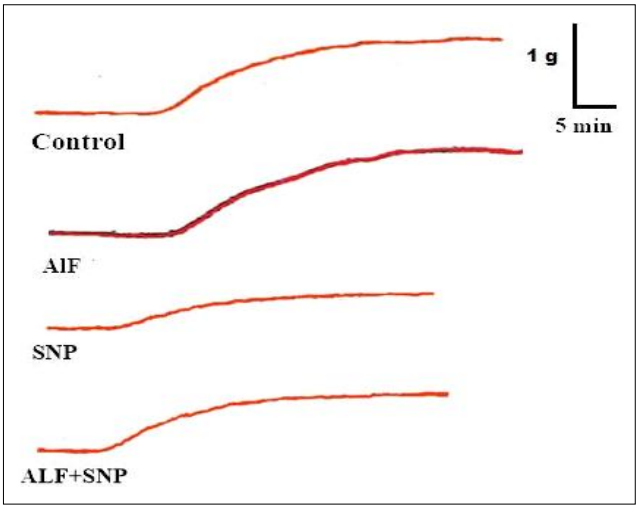

Fig 1: Representative original traces showing CCh (10^{-5} M)-induced contraction, potentiating effect of AlF (10^{-5} M) on CCh-induced contraction, inhibition of CCh-induced contraction by SNP (10^{-5} M) and reversal of the SNP-induced inhibition by AlF (10^{-5} M).

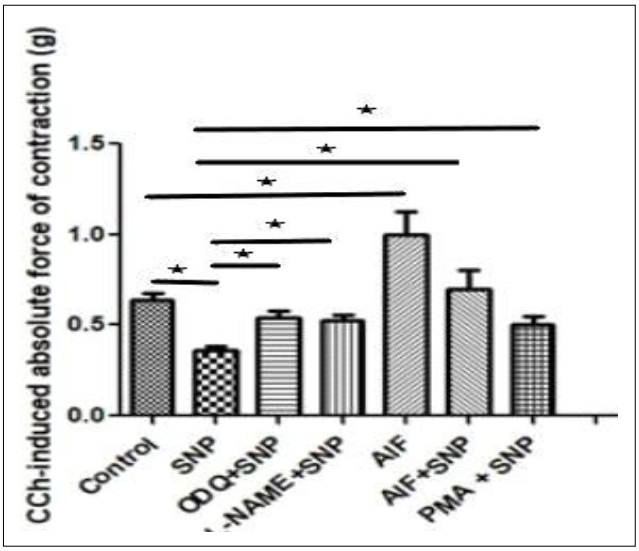

Fig 2: Bar diagram showing inhibition of CCh-induced absolute force of contraction by SNP (10^{-5}M) and its reversal with ODQ(10^{-5} M), L-NAME(10^{-5} M), AlF (10^{-5} M) and PMA (10^{-5} M). Values represent mean±SEM. ($p<0.05$).

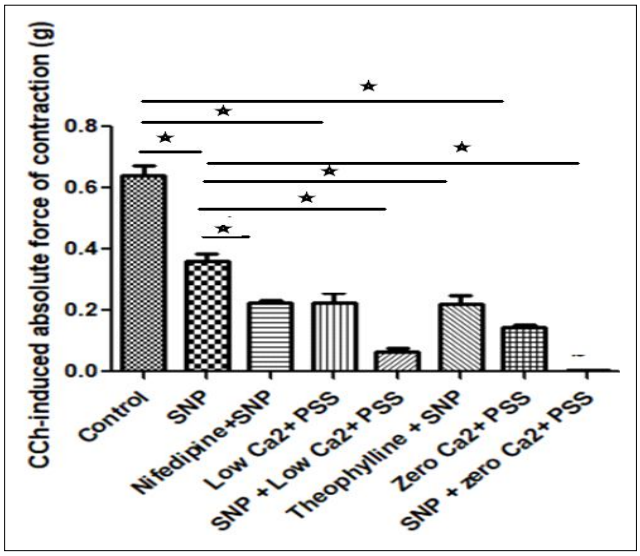

Fig 3: Bar diagram showing inhibition of CCh-induced absolute force of contraction by SNP(10^{-5} M) and a potentiating effect in presence of nifedipine(10^{-5} M), in low Ca^{2+} PSS, in presence of theophylline (10^{-5} M) and in zero Ca^{2+} PSS, respectively. Values represent mean±SEM. ($p<0.05$).

of intracellular Ca^{2+} on SNP evoked inhibition. CCh (10^{-5} M)-induced contraction was obtained first in absence and presence of SNP (10^{-5} M) and then in the presence of theophylline (10^{-5} M) plus SNP in normal PSS. Theophylline potentiated the SNP evoked inhibition (Fig 3). Thus, the absolute force of contraction in presence of SNP and theophylline plus SNP was 0.36±0.025 g (n = 22) and 0.22±0.03 g (n = 4), respectively ($p<0.05$).

Reduction of both spontaneous contractility and CCh-induced contraction by methyl xanthine has been reported to be due to decrease in intracellular Ca^{2+} through blockade of PDE in guinea pig DSM (Xin *et al.*, 2012). Thus, the potentiating effect of theophylline on SNP mediated inhibition also indicates inhibition of intracellular Ca^{2+} release.

In another set of experiments, CCh (10^{-5} M) - induced contraction was first obtained in normal PSS and then in zero Ca^{2+} PSS (Ca^{2+} was replaced with equimolar concentration of strontium, Sr^{2+}). Following this, DSM strips were incubated with SNP (10^{-5} M) in zero Ca^{2+} PSS and then again subjected to CCh-induced contraction. In zero Ca^{2+} PSS, CCh-elicited contraction was inhibited to 0.14±0.01 g (n = 4). Interestingly, there was total inhibition of CCh-induced contraction by SNP in zero Ca^{2+} PSS (Fig 3). Sr^{2+} has been reported to substitute for extracellular Ca^{2+} which supports muscle contractions that utilize influx of extracellular Ca^{2+}, while inhibiting contractions that depend on release of intracellular Ca^{2+}. Sr^{2+} also blocks action mediated by Ca^{2+} release while it maintains effects mediated by influx of extracellular Ca^{2+} (An *et al.*, 2002). Therefore, as observed in the present study, the inhibitory effect of SNP is due to inhibition of Ca^{2+} influx with resultant inhibition of intracellular Ca^{2+}. SIN-1 and SNP earlier inhibited Ca^{2+}-induced concentration-contraction curve in K^{+}-depolarised solution indicating inhibition of intracellular Ca^{2+} release (Vijayraj *et al.*, 2011).

CONCLUSION

Taken together, from the results of the present study it can be concluded that i) the inhibitory effect of SNP in CCh-induced contraction in goat DSM is both NO-dependent and cGMP-dependent. ii) SNP inhibits G-protein coupled PKC leading to inhibition of both Ca^{2+} entry and intracellular Ca^{2+} release with a resultant decrease in intracellular Ca^{2+}.

Conflict of interest: None.

REFERENCES

An J.Y., Yun H.S., Lee Y.P., Yang S.J., Shim J.O., Jeong J.H., Shin C.Y., Kim J.H., Kim D.S. and Sohn U.D. (2002). The intracellular pathway of the acetylcholine-induced contraction in cat detrusor muscle cells. British Journal of Pharmacology. 137: 1001-1010.

Barua, R.K., Deka, D.K., Ahmed, N. and Sarma, S. (2010). Nitric oxide produces inhibitory effects against carbachol-induced contraction in detrusor muscles of goat. Indian Journal of Animal Sciences. 80(2): 110-112.

Chung, B.H., Seung, K.C. and Ki, C.C. (1996). Effects of nitric oxide on detrusor relaxation. Journal of Urolog. 155: 2090-2093.

Dhruva, A., Hamsavardhini, V.K. and Peedicayil, J. (2019). Avanafil inhibits the contractility of the isolated caprine detrusor muscle. Internal Journal of Applied and Basic Medical Research. 9(4): 231-234.

Fujiwara, M. andersson, K.E. and Persson, K. (2000). Nitric oxide-induced c-GMP accumulation in the mouse bladder is not related to smooth muscle relaxation. European Journal of Pharmacology**.** 401: 241-250.

Griffith, T.M. (1994). Modulation of blood flow and tissue perfusion by endothelium-derived relaxing factor. Exp.erimental Physiology**.** 79: 873-913.

Hypolite, J.A. and Malykhina, A.P. (2015). Regulation of urinary bladder function by protein kinase C in physiology and pathophysiology, BioMed Central Urology. DOI: 10.1186/s12894-015-0106-6.

James, M.J., Brimingham, A.T. and Hill, S.J. (1993). Relaxation of human isolated detrusor strips in reponse to electrical field stimulator: A possible role for nitric oxide in human bladder. British Journal of Clinical Pharmacology. 35: 366-372.

Jezior, J.R., Brady, J.D., Rosenstein, D.I., Mc Cammon, K.A., Miner, A.S. and Ratz, P.H. (2001). Dependence of detrusor contractions on calcium sensitization and calcium entry through LOE-908-sensitive channels. Br J. Pharmacol. 134: 78-87.

Moon, A. (2002). Inûuence of nitric oxide signaling pathways on pre-contracted human detrusor smooth muscle *in vitro.* British Journal of Urology International. 89: 942-949.

Persson, K., Pandita, R.K., Aszòdi, A.M., Ahmad, A.M., Pfeifer, A., Fässler, R. and Andersson, K.E**.** (2000). Functional characteristics of urinary tract smooth muscles in mice lacking cGMP protein kinase type I. American Journal of Physiology Regulatory Integrative and Comparative Physiology. 279(3): R1112-20.

Qui, Y., Kraft, P., Craig, E.C., Liu, X. and Haynes-Johnson, D. (2002). Cyclic nucleotide phosphodiesterases in rabbit detrusor smooth muscle. Urology. 59(1): 145-9. doi: 10.1016/s0090-4295(01)01471-6.

Satake, Y., Satoh, K., Nogi, M., Omura, J., Godo, S., Miyata, S., Saito, H., Tanaka, S., Ikumi, Y., Yamashita, S., Kaiho, Y., Tsutsui, M., Arai, Y. and Shimokawa, H. (2017). Crucial roles of nitric oxide synthases in β-adrenoceptor-mediated bladder relaxation in mice. American Journal of Physiology Renal Physiology. 312: F33-F42.

Schmid, J., M€uller, B., Heppeler, D., Gaynullina, D., Kassmann, M., Gagov, H., Mladenov, M., Gollasch, M. and Schubert, R. (2018). The unexpected role of calcium-activated potassium channels: limitation of NO-Induced arterial relaxation. Journal of American Heart Association. DOI: 10.1161/JAHA.117.007808.

Schneider, T., Fetscher, C., Krege, S. and Michel, M.C. (2004). Signal transduction underlying carbachol-induced contraction of human urinary bladder. Journal of Pharmacology and Experimental Therapeutics. 309: 1148-1153.

Thornbury, K.D., Hollywood, M.A. and McHale, N.G. (1992). Mediation by nitric oxide of neurogenic relaxation of the urinary bladder neck muscle in sheep. Journal of Physiology. 451: 133-44.

Vijayraj, D., Roy, D.C. and Deka, D.K. (2011). Nitric oxide donors inhibit calcium stimulated contraction in detrusor muscle of goat. Indian Journal of Animal Science. 81(12): 1208-1211.

Wang, T., Kendig, D.M., Trappanese, D.M., Smolock, E.M. and Moreland, R.S. (2012). Phorbol 12,13-dibutyrate-induced, protein kinaseC mediated contraction of rabbit bladder smooth muscle. Frontiers in Pharmacology. https://doi.org/10.3389/fphar.2011.00083

Wuest, M., Hiller, N., Braeter, M., Hakenberg, O.W., Wirth, M.P. and Ravens, U. (2007). Contribution of Ca2+ influx to carbachol-induced detrusor contraction is different in human urinary bladder compared to pig and mouse. European Journal of Pharmacology. 565: 180-189.

Xin, W., Cheng, Q., Soder, R.P., Rovner, E.S. and Petkov, G.V. (2012). Inhibition of phosphodiesterases relaxes detrusor smooth muscle *via* activation of large-conductance voltage-and Ca^{2+}- activated K^+ channel. American Journal of Physiology: Renal Physiology. 302(9):1361-1370.