



Nitric oxide donors inhibit calcium stimulated contraction in detrusor muscle of goat

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ABSTRACT

The present study was undertaken to examine the role of nitric oxide on the modulation of Ca^{2+} in the urinary bladder muscles of goat. CaCl_2 (10^{-5} M – 10^{-2} M) induced cumulative concentration-contraction curves were generated at an increment of 0.5 log unit in isolated detrusor muscle strips in organ bath studies. SNP (sodium nitroprusside) and SIN-1 (3-morpholino-sydnominines) at a concentration of 10^{-6} M and 10^{-5} M were used to study the role of NO on extra cellular Ca^{2+} . Results were compared with a known L-type Ca^{2+} channel blocker, nifedipine (10^{-6} M). In another set of experiments, to study the role of NO on intra-cellular Ca^{2+} , carbachol (CCh, 10^{-4} M) induced sub-maximal contractions of detrusor tissues were produced in Ca^{2+} -free EGTA containing PSS either in the absence or presence of SNP and SIN-1. The present investigation showed that NO donors SIN-1 and SNP concentration dependently shifted the concentration-contraction curves of CaCl_2 . The NO donors also inhibited the CCh-induced contraction. Thus, it can be concluded that SNP and SIN-1 are inhibitors of both extracellular as well as intracellular Ca^{2+} in goat detrusor muscle.

Key words: Calcium, detrusor, Nitric oxide, SIN-1, SNP

Nitric oxide (NO) is considered as one of the mediators of non-adrenergic, non-cholinergic (NANC) relaxation in smooth muscle from many organs with an inhibitory role in their autonomic regulation (Sneddon and Graham 1992). NO mediated relaxation of urinary bladder muscle was reported in sheep (Thornbury *et al.* 1992), and dog urethra (Hashimoto *et al.* 1993). Photo-activated release of NO from a donor can relax the detrusor in rat (Chung *et al.* 1996). In contrary, NO donors were reported to have excitatory role in bladder muscles of guinea pig (Moon 2000) and mouse (Fujiwara *et al.* 2000). At the same time, NO donors evoke a complex response in pre-contracted human detrusor (Moon 2002). NO donor, sodium nitroprusside (SNP) produces an inhibitory effect against carbachol-induced contraction in goat detrusor smooth muscle (Barua *et al.* 2010).

Increase in the intracellular Ca^{2+} concentration is the key process required for the activation of contraction in bladder smooth muscle (Brading 1992). However, debate remains as to whether this increase is due to influx from the extra cellular space and / or release from intracellular stores. Nakayama (1993) has highlighted the importance of extra

cellular Ca^{2+} in response to ATP in guinea pig; while acetylcholine was shown to cause inositol triphosphate (IP_3)-induced release from intracellular stores (Noronha-Blob *et al.* 1989). In rabbit detrusor strips, ATP and acetylcholine were proposed to cause either/ both extracellular Ca^{2+} influx and release from stores (Kishi *et al.* 1992).

Keeping these facts in mind as well as the inhibitory effect of an NO donor SNP on CCh-induced contraction on goat detrusor muscle, we wanted to examine the role of nitric oxide in the Ca^{2+} channel modulations in the urinary bladder muscles of goat.

MATERIALS AND METHODS

Urinary bladders of adult Assam local goat of either sex slaughtered in local abattoirs were collected in cold aerated modified Krebs's solution and brought to the laboratory. The neck and dome parts of the bladders were removed and the bladders were cut open with the help of a fine scalpel in petri-dish containing aerated physiological solution (Composition in mM : NaCl 120, KCl 5.9, MgCl_2 1.2, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5, NaHCO_3 15, NaH_2PO_4 1.2, Glucose 11). The fascia and urothelium of the urinary bladders were removed and smooth muscle strips of about 2–3 mm breadth and 4–5 mm length were dissected out. The strips were tied on either ends with thread, mounted into a thermostatically controlled ($37 \pm 0.5^\circ\text{C}$) organ bath (20 ml capacity)

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containing physiological solution and were continuously aerated. The tissues were then equilibrated under a resting tension of 1.0 g for a period of 60 min. During this period the bathing fluid was changed, every 15 min. Isometric contractions were recorded by means of a force displacement transducer connected to a single channel physiograph. After equilibrating the tissue strips for 1 hr, following sets of experiments were used:

In this series of experiments, the tissues were first equilibrated in Ca^{2+} free EGTA containing PSS (composition in mM: NaCl 118, KCl 4.7, MgCl_2 1.0, Tris 12.0, glucose 5.55 and EGTA 100.0) for 1 h followed by Ca^{2+} free EGTA containing K^+ (80 mM) depolarizing solution for 30 min. The tissues were then washed several times with nominally Ca^{2+} free (zero EGTA) K^+ - depolarizing solution for 15 min during which phenoxybenzamine (10^{-6} M) was continuously present. Cumulative concentration-contraction curves of CaCl_2 were generated at an increment of 0.5 log unit either in the absence or presence of SNP or SIN-1. Number of samples for each set of experiment was 4. Results were compared with a known L-type Ca^{2+} channel blocker, nifedipine. The contractile responses to Ca^{2+} were expressed as percent maximum response elicited at CaCl_2 (10^{-2} M) in the absence of any blocker. Tris buffer (12 mM) was used in K^+ depolarizing saline solution to prevent precipitation of Ca^{2+} at higher concentrations.

In order to see the role of NO in agonist induced contractions utilizing intracellular Ca^{2+} , the tissues were subjected to sub maximal contraction with carbachol (CCh, 10^{-4} M) following 60 min equilibration. When the contraction reached a steady state, the tissues were washed with Ca^{2+} free EGTA containing PSS and were incubated for 1 h. Subsequently, the tissues were subjected to repeated submaximal contraction with CCh (10^{-4} M) at 30 min interval to see any alteration in the amplitude of contractions either in the absence or presence of SNP or SIN -1. In this series of experiments also, number of samples for each set of experiment was 4.

The results (absolute force/% contractions) are presented as mean $\pm$ SEM and student's 't' test was employed to measure the level of significance. The EC_{50} values were determined by regression analysis and were expressed as geometric mean with their 95% confidence limits (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The comparative effects of SNP and a prototype calcium channel blocker, nifedipine on Ca^{2+} induced contraction in K^+ (80 - mM) depolarized goat urinary bladder muscles are shown in Fig. 1. CaCl_2 (10^{-5} - 10^{-2} M) added cumulatively at an increment of 0.5 log unit elicited concentration related contractions of detrusor muscle with an EC_{50} of 3.95×10^{-4} M (95% CL, 3.819 – 4.10×10^{-4} M). The force generated at the highest concentration of CaCl_2 used in the present

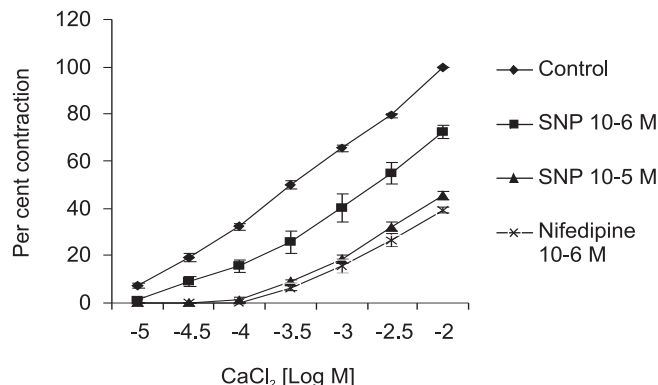


Fig. 1. Effects of SNP (10^{-6} or 10^{-5} M) and nifedipine (10^{-6} M) on Ca^{2+} induced contraction in K^+ (80 mM) depolarized goat detrusor strips. Values are expressed as mean $\pm$ SEM.

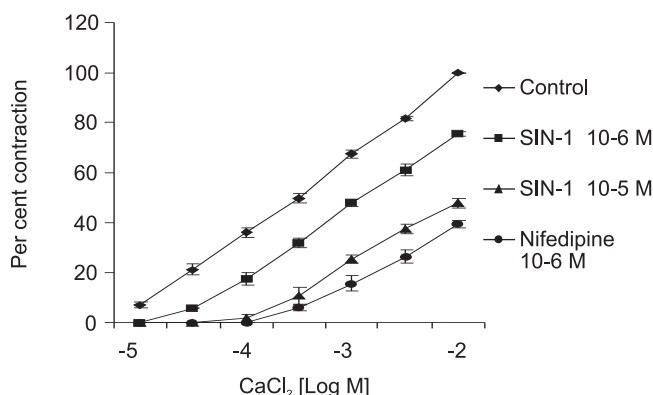


Fig. 2. Effects of SIN-1 (10^{-6} or 10^{-5} M) and nifedipine (10^{-6} M) Ca^{2+} induced contraction in K^+ (80 mM) depolarized goat detrusor strips. Values are expressed as mean $\pm$ SEM.

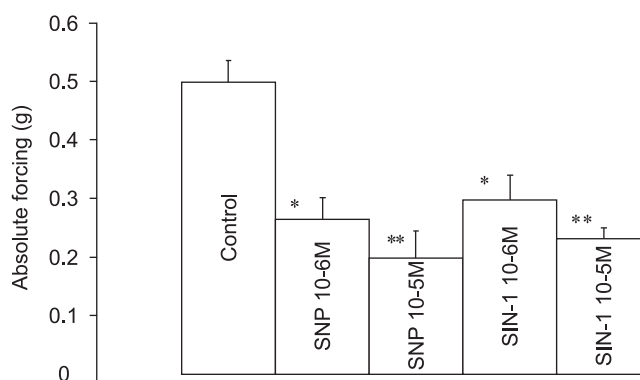


Fig. 3. Comparative effects of SIN-1 (10^{-6} or 10^{-5} M) and SNP (10^{-6} or 10^{-5} M) on CCh (10^{-4} M) induced contractions in goat detrusor tissues in Ca^{2+} -free (plus EGTA) PSS. Values are expressed as mean $\pm$ SEM. (* $P < 0.01$ and ** $P < 0.05$).

experiment was 1.38 ± 0.04 g. Pretreatment of the tissues for 30 min with SNP (10^{-6} or 10^{-5} M) caused a rightward shift in the concentration-contraction curve of Ca^{2+} (10^{-5} - 10^{-2} M) with a corresponding increase in its EC_{50} . Thus, the EC_{50} values in presence of SNP at 10^{-6} and 10^{-5} M were 3.12×10^{-3} M (95% CL, 0.45 – 2.18×10^{-3} M) and 5.73×10^{-2} M

(95% CL, $4.54 - 7.25 \times 10^{-2}$ M) respectively.

The comparative effect of SIN-1 and calcium channel blocker nifedipine on Ca^{2+} induced contractions in goat detrusor muscle is shown in Fig. 2. The force generated at the highest concentration of CaCl_2 was 1.21 ± 0.047 g and the EC_{50} obtained by adding CaCl_2 (10^{-5} - 10^{-2} M) cumulatively at an increment of 0.5 log unit was 3.84×10^{-4} M (95% CL, $3.66 - 4.03 \times 10^{-4}$ M). SIN-1 (10^{-6} or 10^{-5} M) caused a rightward shift in the concentration contraction curve of Ca^{2+} (10^{-5} - 10^{-2} M) with corresponding increase in EC_{50} values. Thus, the EC_{50} values in presence of SIN-1 at 10^{-6} or 10^{-5} M were 5.19×10^{-3} M (95% CL, $5.0 - 5.34 \times 10^{-3}$ M) and 1.68×10^{-2} M (95% CL, $0.22 - 12.8 \times 10^{-2}$ M) respectively.

Pretreatment of the tissues for 30 min with nifedipine (10^{-6} M) produced a right ward displacement of the concentration - response curve of Ca^{2+} with EC_{50} of 6.15×10^{-2} M (95% CL, $5.48 - 6.89 \times 10^{-2}$ M).

Ca^{2+} influx through voltage dependent Ca^{2+} channel plays a major role in mediating contractions in urinary bladder smooth muscle (Rivera and Brading 2006). The inhibitory effect of SIN-1 and SNP on extracellular Ca^{2+} induced contraction of goat detrusor as observed in the present study is in agreement with an earlier observation in goat coronary artery (Deka et al. 2000). SIN-1 also exhibited a predominantly inhibitory effect on Ca^{2+} current in frog cardiac myocytes (Wahler and Dollinger 1995). Similarly, SNP was reported to attenuate Ca^{2+} influx in rat aorta (Magliola and Jones 1990) and to decrease inward Ca^{2+} current in single smooth muscle cells isolated from pulmonary artery (Clapp and Gurney 1991).

There was no alteration in the amplitude of contractions after repeated exposure to CCh (10^{-4} M) in Ca^{2+} free EGTA - containing PSS (Figure not shown). The sensitivity of the CCh (10^{-4} M) - induced contractions to SNP and SIN-1 (10^{-6} or 10^{-5} M) is shown in Fig. 3. CCh produced an absolute force of 0.499 ± 0.037 g in Ca^{2+} - free EGTA- containing PSS. Pretreatment of the tissues with SNP (10^{-6} and 10^{-5} M) for 30 min inhibited the CCh (10^{-4} M) induced contraction by $40.63 \pm 5.98\%$ ($P < 0.05$) and $64.65 \pm 4.45\%$ ($P < 0.01$), respectively. Thus, the forces generated in presence of SNP at 10^{-6} and 10^{-5} M were 0.264 ± 0.038 g, and 0.198 ± 0.047 g, respectively.

Similarly, pretreatment of the tissues with SIN-1 for 30 min also inhibited the CCh induced contractions in goat urinary bladder strips. The % inhibition of CCh induced contraction was $27.52 \pm 5.12\%$ ($P < 0.05$) and $58.05 \pm 3.52\%$ ($P < 0.01$) at 10^{-6} and 10^{-5} M, respectively. Thus, the forces generated by CCh in presence of SIN-1 at 10^{-6} and 10^{-5} M were 0.297 ± 0.043 g and 0.231 ± 0.019 g, respectively.

In the present study, the CCh induced contraction was inhibited by both SNP and SIN-1 in goat detrusor muscle indicating that both are inhibitors of intracellular Ca^{2+} release induced by CCh in goat detrusor muscle. Muscarinic

stimulation and neurally released acetylcholine have been reported to initiate oscillatory increase in $[\text{Ca}^{2+}]_i$ due to both Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum (SR) in urinary bladder muscle (Mostwin 1985, Hashitani et al. 2000). Magliola and Jones (1990) also reported that SNP reduced noradrenaline induced contraction of rat aorta through inhibition of intracellular Ca^{2+} release. Hence, it may be concluded from the present investigation that NO donors SIN-1 and SNP are inhibitors of both extracellular and intracellular calcium in goat detrusor muscle and this might be attributed to the inhibitory effect of the NO donors.

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