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Relaxation of Goat Detrusor Muscle by L-arginine Involves NO-dependent but Guanylyl Cyclase Independent Activation of K_{Ca} Channels

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ABSTRACT

Background: Urinary incontinence is a major problem both in man and animals particularly dogs. L-arginine, the precursor of NO, relaxes coronary artery smooth muscle by opening of K_{ATP} channels. L-arginine has beneficial circulatory effects in patients with essential and secondary hypertension. However, not much is known about the role of L-arginine on bladder physiology. In view of this, the present work investigated the functional role of L- arginine on detrusor smooth muscle of goat.

Methods: Detrusor strips of goat, collected from local abattoir were mounted in a thermostatically controlled (37° ± 0.5°C) organ bath (20 ml capacity) containing physiological solution. After 1 hr of equilibrium, carbachol (CCh) (10^{-5} M) was used to induce sub-maximal contraction. L-arginine (10^{-3} M) was added at the plateau of contraction to see any observable effect in absence and presence of modulators of NO and ion channels.

Result: L- arginine (10^{-3} M) reversed the contractions induced by CCh (10^{-5} M) on detrusor tissues. Methylene blue (MB) (10^{-5} M), the non-specific guanylyl cyclase inhibitor, failed to attenuate the relaxant response of L-arginine but, the NO synthase inhibitor L-NAME (3×10^{-6} M) inhibited the relaxant response of L-arginine. The K_{ATP} channel blocker glibenclamide (10^{-6} M) failed to inhibit the relaxation induced by L-arginine while K_{Ca} channel blocker tetraethylammonium (TEA) (10^{-3} M) inhibited the relaxant response of L-arginine. The results of the present study suggest that L-arginine produces relaxation of goat detrusor muscle and the L-arginine-elicited relaxation is NO-dependent but guanylyl cyclase independent which activates K_{Ca} channels.

Key words: Detrusor, K_{Ca} channels, L-arginine, Nitric oxide.

INTRODUCTION

The nitrergic mechanism on bladder physiology is paradoxical, yet NO is considered as one of the inhibitory mediators in the smooth muscle of many organs (Sneddon and Graham, 1992). NO produces relaxation of urinary bladder muscle in sheep (Thornbury *et al*., 1992), dog urethra (Hashimoto *et al*., 1993) and human urinary bladder (James *et al*., 1993). Photo-activated release of NO from a donor relaxes the detrusor in rat (Chung *et al*., 1996). Contrary to this, NO donors also produce excitatory response 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). In goat detrusor smooth muscle, sodium nitroprusside (SNP), an NO donor produces an inhibitory effect against Carbachol-induced contraction (Barua *et al*., 2010).

K^+ channels play a vital role in regulating membrane potential and cellular excitability in smooth muscles of urinary tract (reviewed by Brading, 1992). Report also suggests that K_{ATP} channels are modulated by NO. In vascular smooth muscle, nitric oxide synthase inhibitors (L-arginine analogues) like L-NNA and L-NMMA inhibit ATP-sensitive K^+ channels, an effect that could be reversed by L-arginine, an essential amino acid (Kontos and Wei, 1996). L-arginine-mediated vasodilatation has been reported to be independent of NO production both *in vivo* (Calver *et al*., 1990) and *in vitro* (Thomas *et al*., 1989 studies. In goat coronary artery, L-arginine mediated vasodilatation is through NO-independent activation of K_{ATP} channels (Deka *et al*., 2009)

L-arginine has beneficial circulatory effects in patients with essential and secondary hypertension (Nakaki *et al*., 1990; Nakaki and Kato, 1994). Exogenous administration of the amino acid markedly decreases blood pressure in these patients. It is generally believed that, the vascular effects of the amino acid are mediated by the vascular smooth muscle cells upon stimulation by inducible nitric

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oxide synthase (Moncada *et al*., 1991). However, it is not known if the amino acid would be of value in the treatment of urinary incontinence, which is a major problem both in man and animals particularly dogs. Therefore, we wanted to examine the role of L-arginine on detrusor muscle with goat as a model.

MATERIALS AND METHODS

Goat urinary bladders were collected from a local abattoir in and around Khanapara within 20-30 minutes of slaughter and put in cold aerated modified Krebs's solution [Composition in 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] and brought to laboratory. The experiments were conducted between the period of August, 2007 and Sept, 2018, in the Department of Pharmacology and Toxicology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam.

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. The fascia and urothelium of the urinary bladders were removed and detrusor smooth muscle (DSM) 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° ± 0.5°C) organ bath (20 ml capacity) 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 minutes. During this period the bathing fluid was changed every 15 minutes. Isometric contractions were recorded by means of a force displacement transducer connected to a single channel physiograph (Biodevices, India). After equilibrating the tissue strips for 1 hr, single dose of carbachol (CCh) (10^{-5} M) was used to produce sustained contraction. At the plateau of contraction, L-arginine (10^{-1}M) was added to see any observable effect of the amino acid on CCh - pre-contracted detrusor strips. Interestingly, L-arginine produced relaxation of the detrusor strips contracted with CCh.

Effect of methylene blue and L-NAME on L-arginine-elicited relaxation

Methylene blue (MB) is a known inhibitor of soluble guanylyl cyclase (GC), an enzyme activated by nitric oxide (Ignarro *et al*, 1986) and therefore, MB (10^{-5} M) was used to see the involvement of guanylyl cyclase on L-arginine-mediated relaxant response on goat DSM. After obtaining L-arginine (10^{-3} M) - elicited control relaxation response and following several washes of the tissues in the bath with PSS, tissues were incubated with MB for 5 min. Thereafter, CCh-induced contraction was induced followed by addition of L-arginine at the plateau of the contraction in the continued presence of MB.

L-NAME is an L-arginine analogue which by inhibiting endogenous NOS decreases the synthesis of NO (Moncada and Higgs, 1995). L-NAME (3 x 10^{-5}M) was used to see the influence of endogenous nitric oxide on L-arginine elicited relaxation of goat DSM. After obtaining L-arginine (10^{-3} M) - elicited control relaxation response and following several washes of the tissues in the bath with PSS, tissues were incubated with L-NAME for 5 min. Thereafter, CCh-induced contraction was induced followed by addition of L-arginine at the plateau of the contraction in the continued presence of L-NAME.

Effect of K^+ channel blockers on L-arginine-elicited relaxation

TEA is a non-specific K_{Ca} channel blocker while glibenclamide is a K_{ATP} channel blocker. In order to examine the involvement of K^+ channels, L-arginine elicited relaxation was obtained in the absence/presence of either TEA or glibenclamide. After obtaining L-arginine (10^{-3} M) - elicited control relaxation response and following several washes of the tissues in the bath with PSS, tissues were incubated with either TEA (10^{-3} M) or glibenclamide (10^{-6} M) for 20 minutes. Thereafter, CCh-induced contraction was induced followed by addition of L-arginine at the plateau of the contraction in the continued presence of either TEA or glibenclamide.

Statistics

The results (Absolute force/percent relaxation) are presented as means ± S.E.M. (n). Students' 't' test (paired) was used to determine the level of significance.

RESULTS AND DISCUSSION

Time matched control contraction response induced by CCh (10 µM) did not change the amplitude of contraction when the tissue remains contracted for 2 hrs (Fig 1). Amplitude of contraction was also not affected on repeated application of CCh (Fig 1b)

Application of L-arginine (10^{-3} M) at the plateau of CCh-induced contraction elicited relaxation on goat DSM. The maximum relaxation produced by L-arginine was 53.03 ± 0.99 % (n = 20, pooled data) when added at the steady state of CCh (10^{-5} M)-induced contraction (absolute force 0.85 ± 0.02g, n = 20, pooled data).

Effect of methylene blue on L-arginine-elicited relaxation

Guanylyl cyclase inhibitor MB had no effect on L-arginine elicited relaxant response on goat DSM (Fig 2a). The per cent relaxation produced by L-arginine in absence and in presence of MB were 55.13 ± 2.05 % (n=4) and 57.75 ± 1.29 % (n=4), respectively. Interestingly, MB reduced the amplitudes of CCh-induced contractions (absolute force 0.66 ± 0.06g, n = 4 in control and 0.47 ± 0.04g, n= 4 in MB treated tissues) (Fig 2b).

MB has been shown to inhibit both NO mediated relaxation and an increase of cGMP in rat detrusor muscle (Chung *et al*., 1996). The fact that MB failed to attenuate L-arginine elicited relaxant response in goat DSM negates the involvement of guanylate cyclase in L-arginine elicited relaxation. But, present study fails to explain the inhibitory effect of MB on amplitude of CCh-induced contraction.

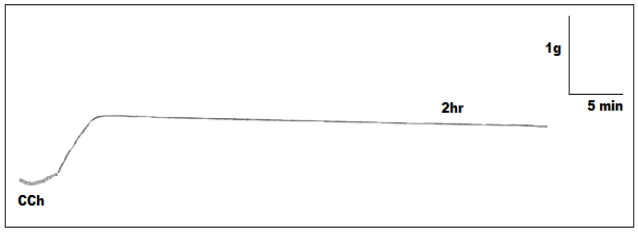

Fig 1a: Original traces showing time matched control contraction response induced by CCh (10 µM) in goat DSM. Note that there is no change in the amplitude of contraction when the tissue remains contracted for 2 hrs.

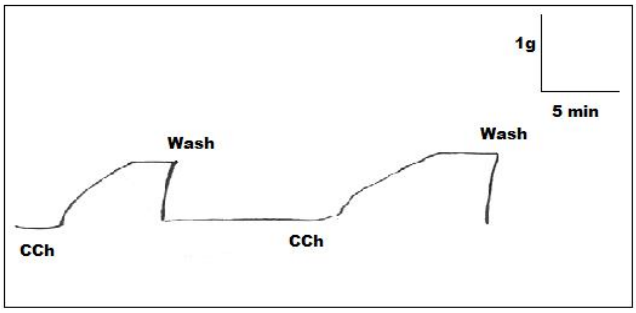

Fig 1b: Original traces showing that repeated application of CCh (10 µM) did not alter amplitudes of contraction in goat DSM.

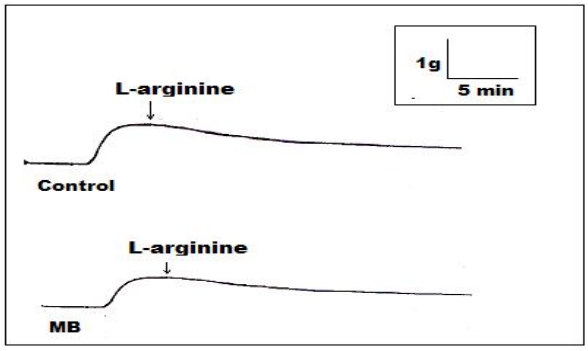

Fig 2a: Original traces showing relaxant response induced by L-arginine (1 mM) on CCh (10^{-5}M) -contracted goat DSM in absence and in presence of MB (10 µM).

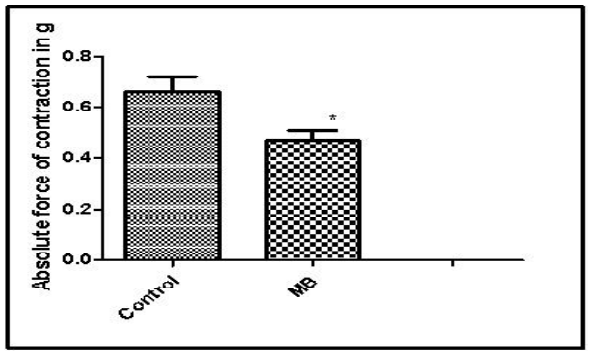

Fig 2b: Bar diagram showing CCh-induced absolute force of contraction (g) on goat DSM in absence (0.66 ±0.06g) and in presence (0.47±0.04g) of MB. Note that the absolute force of contraction was significantly ($p<0.05$) reduced in presence of MB.

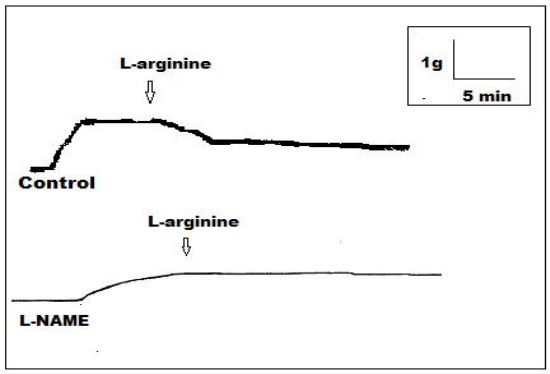

Fig 3a: Original traces showing relaxant response of L-arginine (1 mM) on CCh-contracted detrusor tissues in absence and in presence of L-NAME (3 X 10^{-5}M).

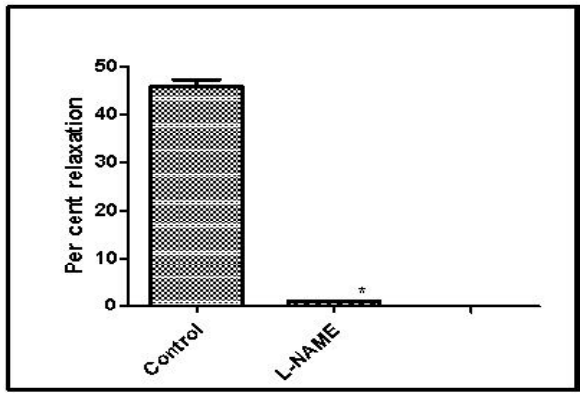

Fig 3b: Bar diagram showing per cent relaxation of L-arginine in control and in presence of L-NAME on CCh-contracted tissues. L-arginine failed to induce relaxation in presence of L-NAME.

Effect of L-NAME on L-arginine-elicited relaxation

L- arginine failed to induce any relaxation when goat DSM were pre-treated with L-NAME (3 x 10^{-5}M) (Fig 3a). Thus, while the per cent relaxation produced by L-arginine was 45.87 ± 1.42 (n=4) in control, it was 0 (Zero) per cent (n=4) in L-NAME treated tissues (Fig 3b). Moreover, L-NAME had no significant effect on CCh (10^{-5} M) - induced absolute force of contraction.

NO was not involved in L-arginine mediated K_{ATP} channel opening in goat coronary artery as L-arginine analogues could not inhibit L-arginine-elicited relaxation (Deka *et al.*, 2009). Contrary to this, activation of K_{ATP} channel was inhibited by pretreatment of porcine coronary smooth muscle cells with L-arginine analogues and it was concluded that K_{ATP} channel activation was due to generation of NO by the essential amino acid (Miyoshi *et al.*, 1994). Interestingly, L-NAME also could inhibit L-arginine elicited relaxation of CCh-contracted goat DSM implicating involvement of NO in L-arginine elicited relaxation in the present study.

Effect of K^+ channel blockers on the relaxant responses of L-arginine in goat detrusor

L-arginine elicited relaxation was inhibited by pre-treatment of goat DSM with TEA (10^{-3} M) for 20 min (Fig 4a). Thus,

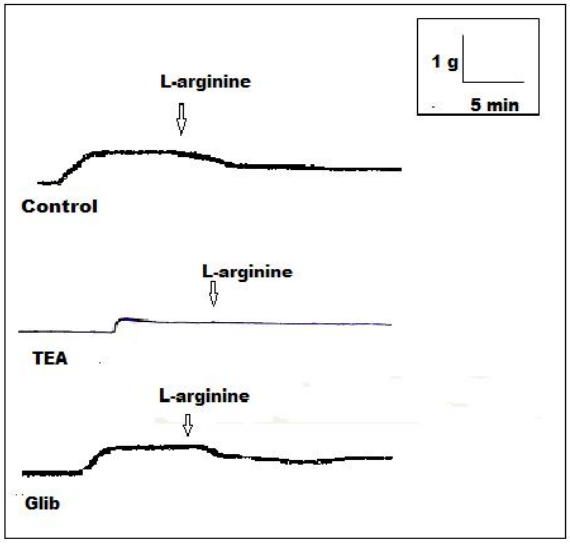

Fig 4a: Original traces showing L-arginine elicited relaxation in absence and in presence of TEA (10^{-3}M) and Glibenclamide (10^{-6} M) on CCh-contracted goat DSM.

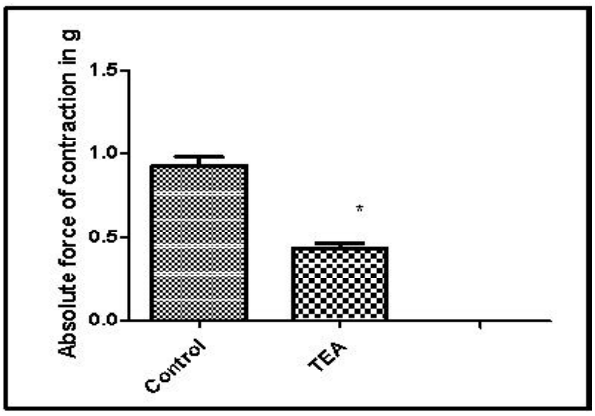

Fig 4b: Bar diagram showing CCh-induced absolute force of contraction (g) on goat DSM in absence and in presence of TEA.

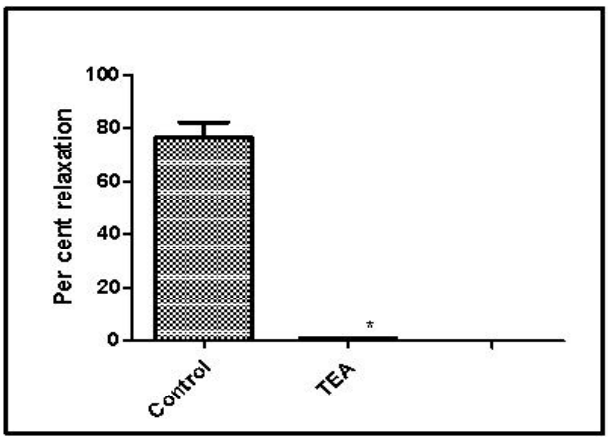

Fig 4c: Bar diagram showing L-arginine elicited relaxation in absence and in presence of TEA. Note that L-arginine failed to induce relaxation in presence of TEA on CCh-contracted tissues.

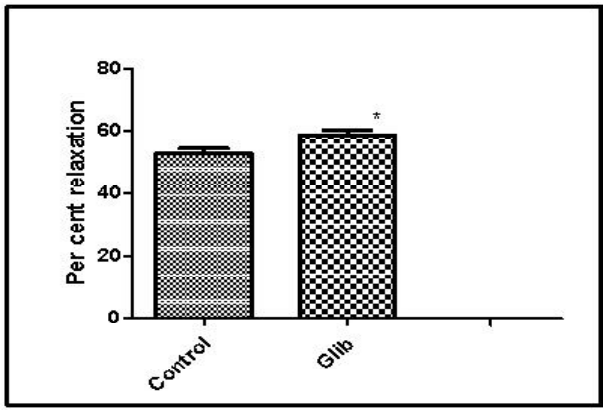

Fig 4d: Bar diagram showing L-arginine elicited relaxation in absence and in presence of glibenclamide. Note that glibenclamide potentiated the L-arginine-elicited relaxation in goat detrusor tissues ($p<0.05$).

while L-arginine evoked a relaxation of 48.5 ± 2.53 % (n=4) in control, it failed to elicit relaxation in TEA treated tissues (Fig 4b). Further, there was a reduction in CCh-induced amplitude of contraction in presence of TEA (absolute force 0.93 ± 0.05g, n=4 in control and 0.43± 0.03g, n=4, in TEA treated tissues, $p \leq 0.001$) (Fig 4c).

Contrary to this, pre-treatment of the tissues with glibenclamide (10^{-6} M) had no effect on either CCh-induced contraction or L-arginine-elicited relaxation. Rather, there was potentiation of L-arginine elicited relaxation in presence of glibenclamide (Fig. 4d). The per cent relaxations elicited by L-arginine in absence and in presence of glibenclamide were 52.75 ± 1.58 % (n=4) and 58.75 ± 1.56 % (n=4), respectively.

TEA has been shown to inhibit L-arginine and bacterial lipopolysaccharide elicited large relaxation in rat isolated aorta (Hall *et al,* 1996) and a role for the involvement of Ca^{2+}- activated K^+ channels in the mechanism of NO-dependent vasodilator action of L-arginine was suggested in rat aorta. On the other hand, L-arginine elicited relaxation was inhibited by K_{ATP} channel blocker; glibenclamide in goat coronary artery suggesting a role for involvement K_{ATP} channels in L-arginine mediated relaxant response in coronary artery (Deka *et al.,* 2009). However, in the present study, glibenclamide failed to attenuate L-arginine elicited relaxation and instead, TEA inhibited the L-arginine-elicited relaxation in CCh-contracted goat DSM. Therefore, L-arginine-elicited relaxation as observed in the present study is suggestive of mediating through opening of K_{Ca} channel in goat DSM. The observation that glibenclamide potentiated the L-arginine-elicited relaxation in detrusor tissue could be best explained by the fact that blocking of K_{ATP} channels would depolarize the smooth muscle tissues leading to an increase in the driving force of the outward K^+ current. Glibenclamide has earlier been reported to cause small depolarizing effect in smooth muscle of rat aorta (Fauaz *et al.,* 2000). K^+ channel blockers like Ba^{2+}, TEA and 4-AP have

been reported to cause an increase in the magnitude of hyperpolarisation to a K_{ATP} channel opener cromakalim in rabbit mesenteric artery (Murphy and Brayden, 1995). Similarly, blocking of K_{Ca} channel with TEA also augmented L-arginine elicited relaxation in goat coronary artery smooth muscle (Deka *et al.,* 2009).

CONCLUSION

In conclusion, the results of the present study suggest that L-arginine produces relaxation in goat DSM. The L-arginine-elicited relaxation is NO dependent, but independent of guanylyl cyclase, which activates K_{Ca} channels.

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