Research in Veterinary Science 95 (2013) 588–593

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Spasmolytic effect of curcumin on goat ruminal artery is endothelium independent and by activation of sGC

J.R. Dash, S.C. Parija *

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Orissa 751003, India

ARTICLE INFO

Article history:
Received 28 July 2012
Accepted 27 April 2013

Keywords:
Goat ruminal artery
Curcumin
s-Guanylatecyclase
L-NAME
ODQ
4-AP

ABSTRACT

The aim of the present work was to study the mechanism of action of curcumin in vasomotion of a physiologically important artery of ruminant i.e. ruminal artery. ACh and SNP were used to study the role of endothelium in relaxation of this artery. Vasorelaxatation by curcumin was studied in a dose dependent manner, on rings precontracted with 5-hydroxy tryptamine and noradrenalin, in presence and absence of L-NAME, 4AP, ODQ and 4AP + ODQ combination. SNP (1 ηM–100 μM) produced a significant relaxation compared to ACh (0.1–100 μM) on 5-HT (10 μM) and NA (10 μM) induced contraction in endothelium intact rings. Curcumin (10 ηM–100 μM) relaxed the vascular rings in dose dependent manner with maximal relaxation up to 20.94% and 13.81% in 5-HT and NA induced contraction, respectively which was potently blocked by ODQ (10 μM) and combination of 4AP and ODQ (10 μM) but 4AP (10 μM) and L-NAME (100 μM) alone could not block the relaxation and interestingly we observed a slight increase in the tension at higher dose of the agonist (>10 μM). Therefore in goat ruminal artery, curcumin at least in part, act via direct activation of sGC mediated cGMP pathway followed by opening of K^+ ion channel. However other mechanisms may not be ruled out.

1. Introduction

The dried ground rhizome of the perennial herb *Curcuma longa* Linn., called turmeric in English, haldi in Hindi and ukon in Japanese, has been used in Asian medicine since the second millennium BC (Brouk, 1975). Curcumin (diferuloylmethane) the principle yellow pigment present in the rhizome of turmeric (*Curcuma longa* Linn.), has a wide array of pharmacological and biological activities. Studies on the safety of *C. longa* and its derivatives in different animal models (Qureshi et al., 1992) have shown that even at high doses turmeric is non-toxic to laboratory animals. Apart from its antioxidant, anti-inflammatory, anti-infectious and anti-carcinogenic properties, curcumin has been shown to target several molecules like growth factors, transcription factors, cytokines etc., that are involved in the etiology of diverse diseases (Foryst-Ludwig et al., 2004; Swarnakar et al., 2005; Shishodia et al., 2007) and useful in patients with hyperhomocysteinemia and cardiovascular diseases (Ramaswami et al., 2004).

There are several reports establishing diversified role of curcumin in vascular smooth muscle function. It potently blocks homocystine induced endothelial dysfunction in porcine coronary artery (Ramaswami et al., 2004). Curcumin-induced relaxation of isolated porcine coronary arteries has been reported to involve the action of nitric oxide (NO), cyclic guanosine monophosphate (cGMP) and adrenergic β-receptors (Xu et al., 2007). Vasomotor activity of curcumin is, α-Ad and β-Ad receptor-dependent and it is net vasoactive effect is concentration and time dependent on microcirculation (Dewar et al., 2011), guanylate cyclase is involved in the protective effects of curcumin in acute endothelium-dependent vasodilator dysfunction induced by high glucose in rat aortic rings (Fang et al., 2009), calcium channel blockade in isolated rabbit jejunum (Gilani et al., 2005), NO-dependent relaxation as well as contraction and NO-independent mechanism of action in vascular smooth muscle of rat aorta (Sasaki et al., 2003) has been reported recently. In this experiment, we studied the vasomotional effect of curcumin and its underlying mechanisms in isolated goat ruminal artery which is lacking any earlier report.

2. Materials and methods

2.1. Materials

5-Hydroxytrypatamine (5HT), acetylcholine (ACh) and Sodium-nitroprusside (SNP) were purchased from Merck, India, Sigma, USA and LOBAchemie, India, respectively. N^G-nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1one (ODQ) and 4-aminopyridine (4-AP), and curcumin were from Cayman chemical Co., USA. All the solutions were prepared fresh in triple distilled water except ODQ which is soluble in dimethyl

* Corresponding author. Tel.: +91 9437356387.
E-mail address: scp4691@yahoo.co.in (S.C. Parija).

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http://dx.doi.org/10.1016/j.rvsc.2013.04.029

sulfoxide (DMSO). Curcumin stock solution was prepared in 0.5 N NaOH and diluted in PBS.

2.2. Preparation of vascular rings

The right ruminal artery was traced from main celiac artery supplying to the right ventral and dorsal sac of rumen. Ruminal artery (4–5 cm long) was carefully dissected out from the rumen wall towards the anterior end before its bifurcation, as per anatomical description of Wesley and Alvin, 1969. The ruminal artery collected in Modified Krebs–Hanseleit solution (MKHS) from freshly slaughtered goat was removed and dissected from surrounding fat and connective tissues and cut into uniform rings of 2.5 mm length. The arterial rings were prepared carefully so that the endothelium was not damaged. Rings were mounted between two hooks attached to an isometric force transducer sensitive to 5 mg–25 g (Model: MLT 0201, AD instruments, Australia) and kept in a thermostatically controlled organ bath (37 ± 0.5 °C) of 20 mL capacity (Panlab S.I, Spain), containing MKHS (pH 7.4) continuously bubbled with carbogen (5% CO_2 and 95% O_2) and tension was recorded using 8/32 power lab data acquisition system (AD Instruments, Australia) with Labchat6 pro software. MKHS contained the following (in mM): 118.0 NaCl; 4.7 KCl; 2.5 $CaCl_2.2H_2O$; 1.2 $MgSO_4.7H_2O$; 1.2 KH_2PO_4; 11.9 $NaHCO_3$ and 11.1 glucose. The vascular rings were kept under resting tension of 2 g and allowed 90 min for equilibration with continuous washing with Krebs solution in every 15 min interval before starting the experiment (Kathirvel et al., 2010).

2.3. Functional study

After the equilibration period, endothelium intact vascular rings perfused in PSS, pre contracted with sub maximal concentration of 5HT, 10 μM or NA, 10 μM were relaxed with ACh (0.1–100 μM), SNP (1 ηM–100 μM) and Curcumin (10 ηM–100 μM) in a cumulative manner with 0.5 log unit increment for ACh and 1 log unit increment for SNP and Curcumin respectively, in presence or absence of L-NAME, 100 μM, 4-aminopyridine, 10 μM, ODQ, 10 μM and combination of 4-AP and ODQ, 10 μM, pre incubated for 30 min. Vasodilatation effect were expressed as the % of maximal response.

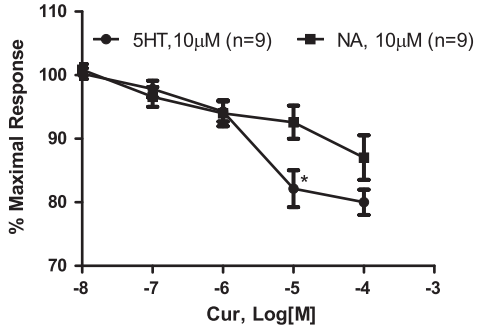


Fig. 1. Concentration – response curve of relaxation induced by curcumin (10 ηM–100 μM) on isolated goat ruminal artery pre-contracted with 5HT, 10 μM and NA, 10 μM. n = number of experiment, $^*P < 0.05$. Cur = Curcumin, 5HT = 5-hydroxy tryptamine, NA = noradrenalin.

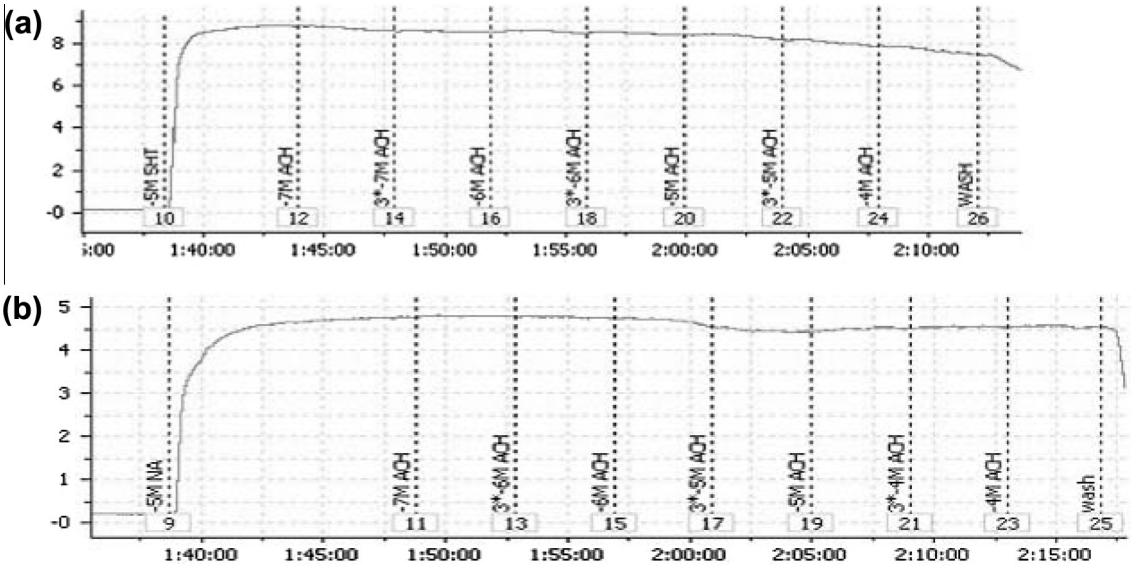
2.4. Statistical analysis

All data was presented as mean ± SEM (standard errors of mean) of measurements in "n" experiments. One way analysis of variance (one way ANOVA) was conducted for comparison between the groups. Dunnett test was used to compare the experimental groups with the control and Turkey's test and unpaired student's "*t*" test was carried out where ever necessary. The responses were expressed as the percentage of reduction of the contraction induced by 5HT or NA. pD_2 value and maximal relaxation (E_{max} or E_{Bmax}) to agonists or antagonist, respectively, was determined for each ring by fitting individual concentration (agonist) – response data to a non-linear sigmoid regression curve in GraphPad prism5 software (GraphPad Prism5, GraphPad Software Inc., San Diego, CA, U.S.A). A '*p*' value < 0.05 was considered statistically significant.

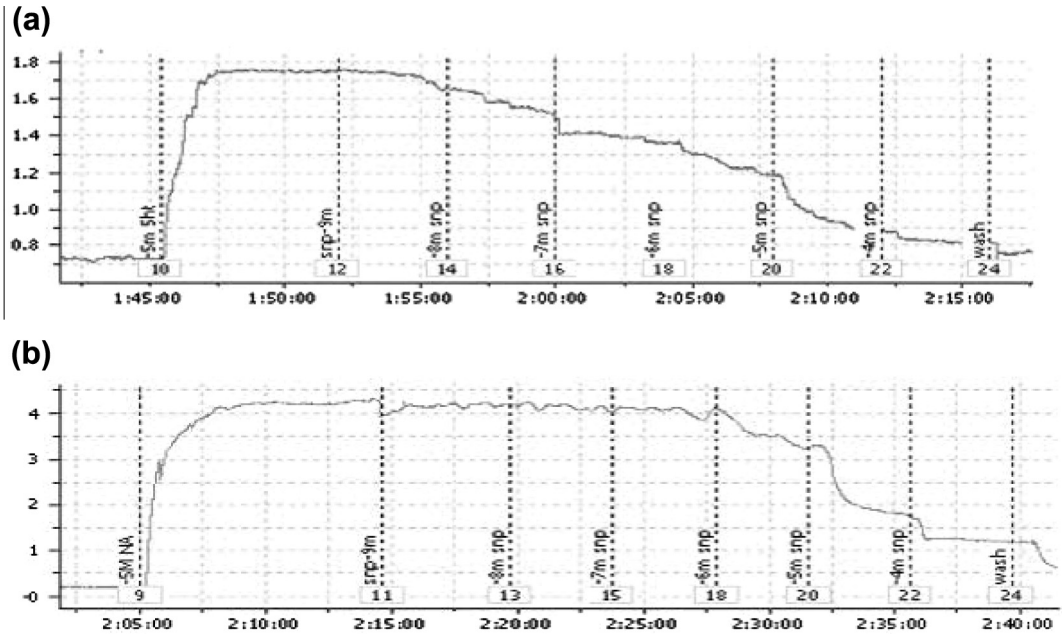
3. Results

3.1. Effect of curcumin on 5HT (10 μM) or NA (10 μM) induced contraction in goat ruminal artery

Curcumin (10 ηm–100 μM) relaxed, the contractions induced by either 5HT (10 μM) or NA (10 μM) in endothelium-intact



Tracing 1. a and b. ACh(0.1–100 μM) induced relaxation in 5HT, 10 μM and NA, 10 μM induced contraction.



Tracing 2. a and b. SNP(1 ηM–100 μM) induced relaxation in 5HT, 10 μM and NA, 10 μM induced contraction.

vascular rings, in dose dependent manner, with EC_{50} value 2.68 μM and 10.58 μM, respectively. There was no significant difference in the response at lower doses but a significant difference was observed at a dose of 10 μM (Fig. 1, Tracing 3a and b).

3.2. Vasorelaxation by ACh, Curcumin and SNP on 5HT (10 μM) or NA (10 μM) induced contraction

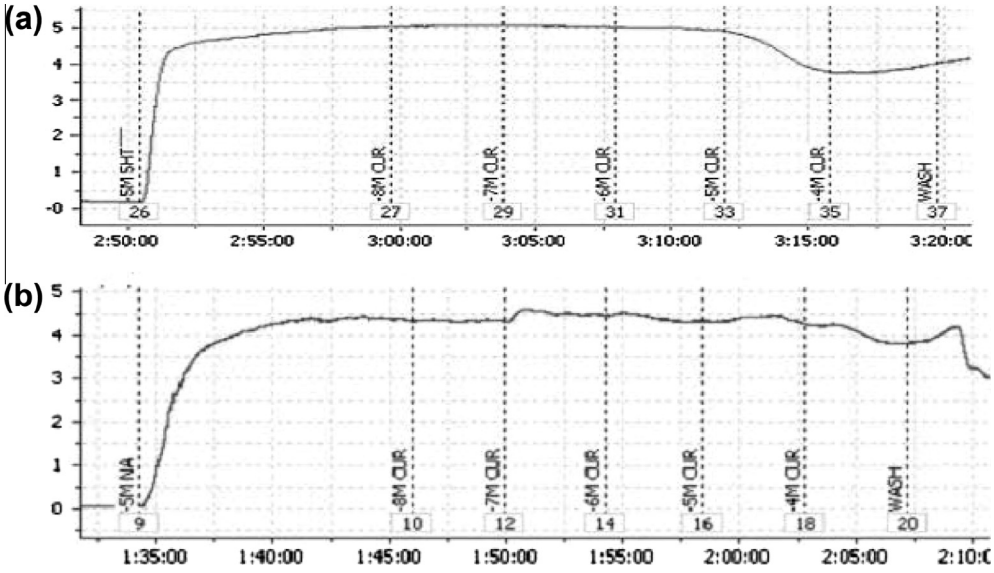
ACh (0.1–100 μM) (Tracing 1a and b), Curcumin (10 ηM–100 μM) (Tracing 3a and b) and SNP (1 ηM–100 μM) (Tracing 2a and b), relaxed the endothelium intact vascular rings in dose dependent manner, with EC_{50} value 6.63 μM, 2.68 μM and 0.89 μM, respectively and 30.2 μM, 10.58 μM and 2.3 μM, respectively, on 5-HT, 10 μM and NA, 10 μM induced contraction (Fig. 2 and 3). The maximal relaxation produced by them were 26.98%, 20.94%, 78.37% and 29.79%, 13.81%, 61.54% on 5HT and NA induced contraction, respectively. pD_2 value and maximal relaxation (E_{max}) are presented as mean ± SEM (Table 1).

3.3. Effect of 4-AP, ODQ and 4AP+ODQ on vasorelaxation by curcumin

4AP (10 μM) had no significant effect on mean maximal response in 5HT, 10 μM induced contraction but increased the EC_{50} value from 10.58 μM to 0.121 ηM in NA, 10 μM induced contraction. ODQ (10 μM) significantly blocked both. 4AP and ODQ (10 μM) combined; potently attenuated the relaxation of both 5HT (10 μM) and NA (10 μM) pre-contracted rings (Fig. 4 and 5). pD_2 value and maximal relaxation (E_{max}) are presented as mean ± SEM (Table 2).

3.4. Effect of L-NAME on vasorelaxation by curcumin

Pretreatment with L-NAME, 100 μM for 30 min had decreased the EC_{50} value from 2.44 μM to 0.25 μM, on 5HT (10 μM) induced contraction but increased the EC_{50} from 10.58 μM to 9.65 ηM in NA (10 μM) induced contraction. L-NAME induced initial decrease then increase in tension of the ring, at dose 10 μM and 100 μM of



Tracing 3. a and b. Curcumin (10 ηM–100 μM) induced relaxation in 5HT, 10 μM and NA, 10 μM induced contraction.

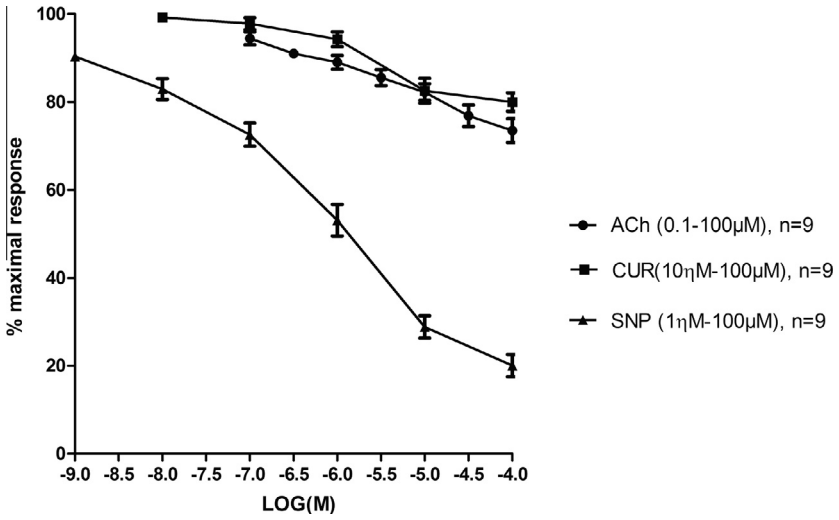


Fig. 2. Concentration – response curves of relaxation induced by ACh, 0.1 μM–100 μM, curcumin, 10 ηM–100 μM and SNP, 1 ηM–100 μM on vascular rings pre-contracted with 5HT, 10 μM. *n* = number of experiment, Cur = Curcumin, ACh = acetylcholine, SNP = sodium nitroprusside.

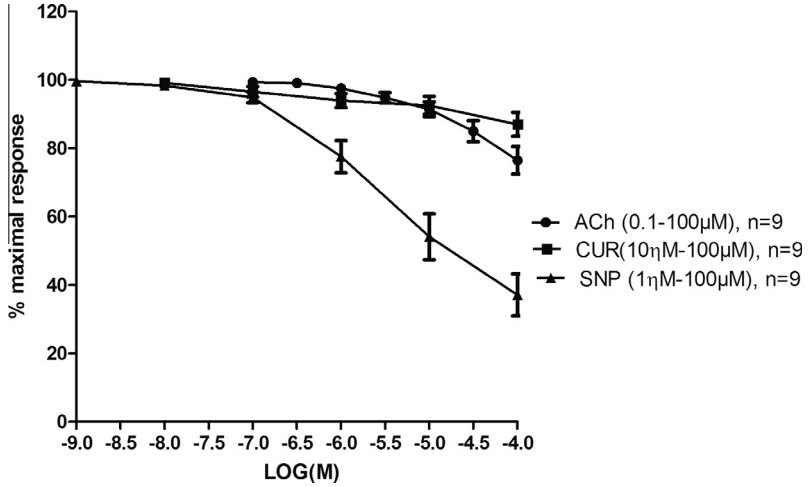


Fig. 3. Concentration – response curves of relaxation induced by ACh, 0.1 μM–100 μM, curcumin, 10 ηM–100 μM and SNP, 1 ηM–100 μM on vascular rings pre-contracted with NA, 10 μM. *n* = number of experiment, Cur = Curcumin, ACh = acetylcholine, SNP = sodium nitroprusside.

Table 1
P_2^D and E_{max} value of ACh (0.1 μM–100 μM), SNP (1 ηM–100 μM) and curcumin (10 ηM–100 μM) induced relaxation on endothelium intact goat ruminal artery rings precontracted with 5-hydroxy tryptamine (5-HT), 10 μM; noradrenalin (NA), 10 μM.

	ACh (n = 9)	CUR (n = 9)	SNP (n = 9)
5HT(10 μM)			
P_2^D	5.179 ± 0.21[a]	5.572 ± 0.23[a,b]	6.048 ± 0.09[a,b]
E_{max}	73.02 ± 2.07[a]	79.06 ± 1.88[a,b]	21.63 ± 2.29[a,b]
NA(10 μM)			
P_2^D	4.52 ± 0.24[a,d]	4.98 ± 0.53[a,c,d]	5.63 ± 0.16[a,c]
E_{max}	70.21 ± 5.49[a]	86.19 ± 3.31[a]	38.46 ± 4.14[a]

P_2^D = Negative logarithm of concentrations of agonist producing 50% of the maximal response.
E_{max} = maximal response by the agonist. P_2^D and E_{max} value were determined by linear regression analysis using GraphPad Prism 5.0 software.
[a] Significant difference between the groups at the level of $p < 0.001$.
[b] Significant difference between the groups at the level of $p < 0.001$.
[c] Significant difference between the groups at the level of $p < 0.01$.
[d] Significant difference between the groups at the level of $p < 0.05$. Values are mean ± SEM, n = no of experiments.

the agonist (i.e. curcumin) (Fig. 4 and 5). pD_2 value and maximal relaxation (E_{max}) are presented as mean ± SEM (Table 2).

4. Discussion and Conclusions

It is known that relaxation of vascular smooth muscle is induced by prostaglandin I_2, Ca^{+2} antagonists, α-blocker, β-blocker (Luscher et al., 1992) in addition to NO (Palmer et al., 1987). In present study, we examined the vasomotional effect of curcumin and found that curcumin (10 ηm–100 μM) relaxed; the endothelium intact vascular rings in dose dependent manner, maximal relaxation was 20.94% and 13.81% in 5-HT (10 μM) and NA (10 μM) induced contraction, respectively. Significant relaxation was found at concentration of agonist = 10 μM.

It is well established that endothelium has obligatory role in vasorelaxation by acetylcholine (Furchgott and Zawadski, 1980). Acetylcholine produces vasodilatation through the endothelial synthesis and release of EDRF (NO). NO, weather released endogenously mediated by acetylcholine receptor or produced intracellular in the smooth muscle cells from the metabolism of nitro

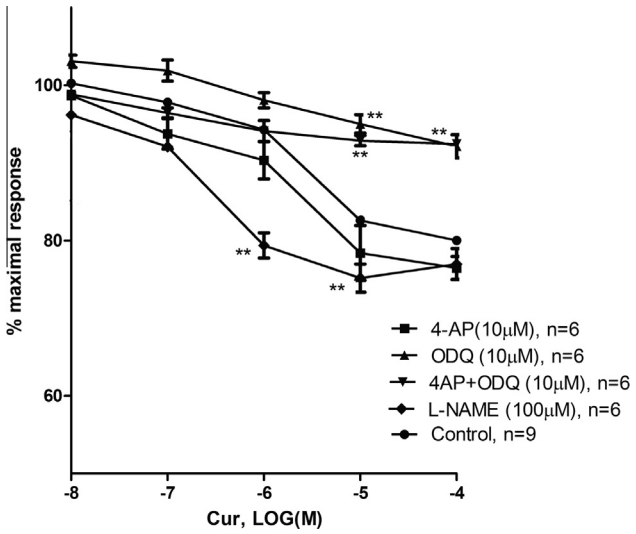


Fig. 4. Concentration – response curves of relaxation induced by curcumin, 10 ηM–100 μM on isolated goat ruminal artery rings pre-contracted with 5HT, 10 μM in presence of 4AP, 10 μM; ODQ, 10 μM; 4AP + ODQ, 10 μM and L-NAME, 100 μM. n = number of experiments, $^{**}p < 0.01$, Control = Curcumin, 4-AP = 4-amino pyridine, ODQ = 1-H-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one, L-NAME = N^G-nitro-L-arginine-methyl ester .

vasodilators like SNP (Kowaluk et al., 1992), activates vascular smooth muscle soluble guanylate cyclase (sGC) to produce an increase in cGMP level (Kowaluk et al., 1992) exerts most of its cellular responses by binding and activating protein kinase G which then phosphorylates intracellular target proteins. In some tissues cellular responses can also occur following either activation or inhibition of cGMP-regulated ion channels (Wanstall et al., 2005). Xu et al., 2007 has reported a significant relaxation of porcine coronary artery by curcumin which involve the vascular endothelium and mediated by nitric oxide (NO), cyclic guanosine monophosphate (cGMP) and adrenergic beta receptor. But in goat ruminal artery it is observed that, ACh (0.1–100 μM) produced maximal relaxation of 26.98%, and 29.79% (E_{max} = 73.02 ± 2.07 and 70.21 ± 5.49) compared to 78.37% and 61.54% (E_{max} = 21.63 ± 2.29 and 38.46 ± 4.14) by SNP (1 ηM–100 μM), on 5HT (10 μM) and NA (10 μM) induced contraction, respectively, suggesting an insignificant role of vascular endothelium in NO mediated vasorelaxation in goat ruminal artery, if present. ODQ is a selective inhibitor of sGC (Garthwaite et al., 1995), acts by binding to heme site of the enzyme (as does NO) and oxidation of the ferrous form of the heme site to the ferric form, which is less sensitive to NO (Schrammel et al., 1996). In our finding, ODQ (10 μM) significantly attenuated the vasorelaxation of curcumin on 5HT and NA induced contraction by 117.24% and 107.04%, respectively (considering the plateu tension of precontracted rings as 100%), shifted the log (agonist) – response curve upward, strongly supports involvement of sGC mediated cGMP in curcumin mediated vasorelaxation in this artery. The effect of curcumin in porcine coronary artery which is potently blocked by nitric oxide synthase (NOS) blockers (Ramaswami et al., 2004; Xu et al., 2007) is not significantly blocked by L-NAME (100 μM) in goat ruminal artery. This finding confirms that there is no significant involvement of endothelium dependent eNOS pathway. Further interestingly a slight increase in tension of the rings, at higher dose of the agonist (>10 μM) (Fig. 4 and 5), may be attributed to activation of adrenergic receptors which has been recently reported by Kathirvel et al. (2010).

We observed that, 4AP (10 μM) alone had no significant effect on mean maximal response of curcumin in 5HT (10 μM) induced contraction but increased the EC_{50} value in NA (10 μM) induced contraction. 4AP and ODQ (10 μM) in combination; potently attenuated the vasorelaxation of both 5HT (10 μM) and NA (10 μM) pre-contracted rings, strongly supports activation of sGC including partial activation of ion channel.

In this study, insignificant relaxation by ACh but significant relaxation by SNP in endothelium intact rings signifies the endothelium independent pathway of relaxation in goat ruminal artery. Significant blockade of relaxation by 4-AP alone, in rings precontracted with NA but not 5HT and no potent blockade but an increasing pattern of tension at higher dose of the agonist in presence of L-NAME alone, in both 5HT and NA precontracted rings, describes no involvement of endothelium dependent NO pathways but partial activation of ion channels which may be mediated by adrenoceptors. Potent blockade by, ODQ alone and in combination with 4-AP, in both ligand system (5HT and NA) confirms active role

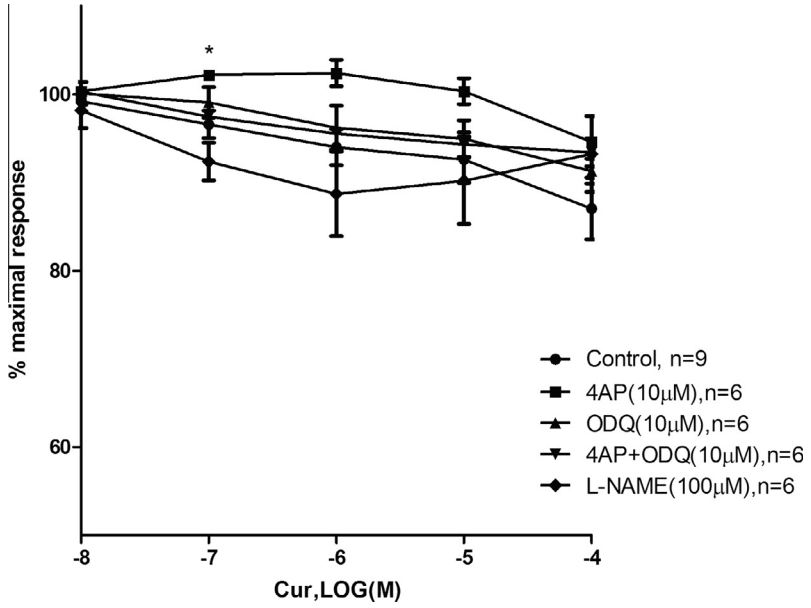


Fig. 5. Concentration-response curves of relaxation induced by curcumin, 10 ηM–100 μM on isolated goat ruminal artery rings pre-contracted with NA, 10 μM in presence of 4AP, 10 μM; ODQ, 10 μM; 4AP + ODQ, 10 μM and L-NAME, 100 μM. n = number of experiments, $^{*}p < 0.05$.

Table 2
$P_2^D/P^{D'_2}$ and E_{max}/E_{Bmax} value of curcumin (10 ηM–100 μM) induced relaxation on isolated goat ruminal artery rings precontracted with 5-hydroxy tryptamine (5-HT), 10 μM; noradrenalin (NA), 10 μM.

	Control, (n = 9)	4AP (n = 6)	ODQ (n = 6)	4AP + ODQ (n = 6)	L-NAME (n = 6)
5HT (10 μM)					
$P_2^D/P^{D'_2}$	5.612 ± 0.19	5.734 ± 0.24^{ns}	5.903 ± 0.26^{ns}	6.779 ± 0.29**	6.586 ± 0.16*
E_{max}/E_{Bmax}	79.16 ± 1.55	75.7 ± 2.05^{ns}	92.81 ± 1.01**	92.74 ± 0.50**	75.58 ± 1.02^{ns}
NA (10 μM)					
$P_2^D/P^{D'_2}$	4.976 ± 0.53	3.917 ± 1.12 ^{ns}	5.77 ± 0.57^{ns}	6.88 ± 0.38^{ns}	8.01 ± 3.52^{ns}
E_{max}/E_{Bmax}	86.19 ± 3.31	86.03 ± 2.29^{ns}	92.26 ± 1.67 ***	94.04 ± 0.67***	90.74 ± 2.28**

Values are mean ± SEM.
n = No of experiments.
^{ns}Not significant ($p > 0.05$).
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

of sGC stimulation with partial opening of ion channels may be involved in vasorelaxation of goat ruminal artery by curcumin. However involvement of other pathways cannot be ruled out which needs further investigation.

Acknowledgement

This work has been approved by institutional animal ethical committee (IAEC) for conducting animal tissue experiment and funded by University research grant. There is no conflict of interest in preparation of this manuscript.

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