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GOAT RUMINAL ARTERY IS MORE SELECTIVE TO EXOGENOUS NITRIC OXIDE DONORS THAN ENDOGENOUS NITRIC OXIDE

JEEVAN RANJAN DASH[1]* AND SUBASH CHANDRA PARIJA[1]
[1]Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar - 751 003, Odisha, INDIA
e-mail: jeevandash5@gmail.com

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***Corresponding author**

ABSTRACT

This study was conducted to study the property of goat ruminal artery towards the exogenous nitric oxidedonor SIN-1 and endogenous nitric oxide generator acetylcholine in NA, 10μM induced contraction. The isolated ruminal arterial rings of goat with intact endothelium were perfused in organ bath and isometric contraction was studied. (1) Noradrenalin (NA), 10μM produced a potent vasotonic response with threshold concentration and E_{max} 3×10^{-7}M and 3×10^{-5}M, respectively (2) 3-morpholinosydnonimine (SIN-1) produced significant relaxation (E_{max} = 38.31 $\pm$ 7.48, EC_{50} = 2.54 μM, n = 6) than acetylcholine (ACh) (E_{max} = 70.21 $\pm$ 5.49, $EC_{50\ max}$ = 30.24 μM, n = 9). (3) L-Arginine preincubation found to have no significant effect (E_{max} = 32.34 $\pm$ 2.72, n = 6) on SIN-I induced relaxation. Similarly L-NAME had no effect. But ODQ potently blocked SIN-I relaxation (E_{max} = 91.04 $\pm$ 8.39, n = 6). From the above observations it is concluded that NO donor SIN-I is a more potent relaxing agent than acetylcholine in goat ruminal artery.The observed effect of SIN-1 in this artery is due to activity of soluble guanylatecyclase and has no prominent NOS (Nitric oxide synthase) activity.

INTRODUCTION

The vascular endothelium regulates the tone of the underlying smooth muscle through the release of potent vasoactive agents. Nitric oxide (NO) has widespread actions as an intra- and inter-cellular signaling molecule that is involved in diverse physiological and pathophysiological mechanisms, including those of the vascular and gastrointestinal smooth muscle (Moncada *et al.,* 1991). Since NO synthase (NOS) is expressed by enteric neurons and NOS inhibitors abolish non-adrenergic non-cholinergic (NANC) relaxation in gastrointestinal smooth muscle, NO is thought to be a mediator of inhibitory neuromuscular transmission in the gut (Sanders and Ward, 1992). Nitric oxide (NO) donors are pharmacologically active substances that, release NO. The discovery of nitric oxide as an endothelium-derived relaxing factor (EDRF) has been established as pharmacological basis for the role of NO donors (Ignarro *et al.,* 1981). It is apparent that the activity of NO, a volatile free radical gas, is not confined to vasodilatation alone. Rather NO has a variety of functions, such as release of prostanoids in infarcted myocardium (Yamamoto *et al.,* 2000), inhibition of platelet aggregation and leukocyte adhesion (Salvemini *et al.,* 1996), production of oxygen free radicals (Freeman *et al.,* 1994). In ruminants ingested fluid subjected to microbial degradation results in volatile fatty acids (VFA) and microbial proteins as end products. Right ruminal artery, a branch of main coeliac artery and its branches supplying the rumen wall has vital role in reticulo-rumen movement and maintenance of blood circulation aiding in proper absorption, distribution of nutrients and maintenance of pH of ruminal fluid (Ruckebusch, 1983). The exact nature of the signal generated by the endothelial cells, the release of EDRF induced by some agents may depend on the species and anatomic sites of the blood vessels. Although NO and NO donors always cause relaxation of vascular smooth muscle via cyclic guanosine 3′,5′-monophosphate (cGMP) accumulation (Moncada *et al.,* 1991) or direct activation of K^+ channels (Bolotina *et al.,*1994), the mechanisms that mediate vasomotor effects of NO vary with the stimulus, the species and the blood vessel under study. Some of the earlier reports have sited that exogenous, but not endogenous nitric oxide inhibits adhesion molecule expression in human endothelial cells (Qian and Fulton, 2012). Exogenous nitric oxide donation causes desensitization of arteriolar relaxing activity in vivo in mice (Ring *et al.,* 2010). Some of the earlier report stated that exogenous nitric oxide can diminish the ruminal contractions, while endogenous NO is not involved in the regulatory mechanism of basal tone and regular phasic contractions of the rumen in healthy sheep (Onaga *et al.*, 2001).To our knowledge the picture in caprine ruminal artery is not clear so far. So the present study was carried out to study the behavior of goat ruminal artery towards endogenous and exogenous nitric oxide.

MATERIALS AND METHODS

Materials

Noradrenaline (NA) and Acetylcholine (ACh) were purchased from Sigma, USA. 3-morpholinosydnonimine (SIN-1), N^G–nitro-L-arginine methyl ester (L-NAME), 1H-[1, 2, 4] oxadiazolo [4, 3-a] quinoxalin-1one (ODQ) were purchased from Cayman chemical, USA. All solutions were prepared fresh before each experiment.

Bioassay procedure

The right ruminal artery was traced from main celiac artery supplying to the right ventral and dorsal sac of rumen. Ruminal artery (4-5cm long) was carefully dissected out from the rumen wall towards the anterior end before its bifurcation, as per anatomical description by Wesley and Alvin, 1969. 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 2.5mm length. The arterial rings were prepared and mounted carefully and given a resting tension of 2g. Vascular response was studied by 8/32 channel Power lab Data acquisition system with isometric force transducers sensitive to 5mg-25g (Model: MLT 0201, AD instruments, Australia). The rings were continuously perfused with MKHS (P^H 7.4) (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 and continuously aerated with carbogen (5%CO_2 and 95%O_2). The rings were left in PSS for 90 min with continuous washing with Krebs solution in every 15 min interval for equilibration before starting the experiment.

Experimental design

After the equilibration period, vascular rings perfused in PSS, pre contracted with sub maximal concentration of NA, 10μM were relaxed with ACh (0.1-100μM) and SIN-1 (ηM-10μM) in a cumulative manner with 0.5 log unit increments for ACh and 1 log unit increment for SIN-I, respectively. Relaxing effect of SIN-1 was also studied in presence of L-NAME, 100μM; L-arginine, 100μM and ODQ, 10μM.

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM) of measurements in 'n' experiments. The relaxant effect were expressed as the percent response (percentage reduction of the maximum contraction induced by NA, 10μM considering plateau tension as 100%). Data were interpreted by fitting individual concentration-response data to a non-linear sigmoidal regression curve and interpolating in Graphpad prism software (Graphpad Prism5, GraphPad Software Inc, San Diego, CA, U.S.A.) and the data were analyzed using one-way analysis of variance (ANOVA) for significant differences between two groups. A level of $p < 0.05$ was accepted as statistically significant.

Table 1: ACh and SIN-I induced relaxation of goat ruminal artery precontracted with noradrenalin (NA), 10 μM

	ACh, (0.1μM-100μM), n = 9	SIN-I, (ηM-10μM), n = 6
$P^D2/P^{D'}2$	4.52 ± 0.23^x	5.59 ± 0.19^y
E_{max}/E_{Bmax}	70.21 ± 5.49^a	38.31 ± 7.48^b

Dissimilar superscripts in a row are significant ($p<0.01$)

Table 2: P^D_2/ $P^{D'}_2$ and E_{max}/E_{Bmax} value of SIN-I (ηM-10μM) induced relaxation of goat ruminal artery precontracted with noradrenalin (NA), 10 μM in presence of agonist and anatagonist

	$P^D2/P^{D'}2$	E_{max}/E_{Bmax}
Control,(n = 6)	5.59 ± 0.19	38.31 ± 7.48
L-Arg, (n = 6)	5.86 ± 0.07	32.34 ± 2.72
L-NAME,(n = 6)	5.37 ± 0.15	36.11 ± 7.17
ODQ, (n = 6)	$5.36 \pm 1.37^*$	$91.04 \pm 8.39^{***}$

*** = $p<0.001$, * = $p< 0.05$; n = no of experiments. P^D_2 = Negative logarithm of concentrations of agonist producing 50% of the maximal response. $P^{D'}2$ = Negative logarithm of concentrations of agonist producing 50% of the maximal response in presence of antagonist. E_{max} = maximal response by the agonist. E_{Bmax} = maximal response by the agonist in presence of antagonist.

RESULTS

Effect of ACh and SIN-1 in goat ruminal artery

Effect of ACh (0.1-100μM) in inducing vasorelaxation of goat ruminal arterial rings on NA (10μM) induced sustained contraction was very negligible (Tracing 2). SIN-1 (Tracing 3) was a better relaxing agent than ACh (see Fig. 1, Table 1).

Role of NOS

30 minutes pre-incubation of L-NAME, 100μM (E_{Bmax} = 36.11 ± 7.17%) and had no significant effect on SIN-I (1ηM-10μM) relaxation (E_{max} = 38.31 ± 7.49%) implied that eNOS was not activated by SIN-1. Adding L-arginine, 100μM also did not augment the SIN-1 effect.

Role of sGC

Rings incubated with ODQ, 10μM for 30mins potently blocked (E_{Bmax} = 91.04 ± 8.39%) the vasorelaxation by SIN-1.

DISCUSSION

In the present study NA (0.1-100 μM) produced potent vasotonic response in goat ruminal artery (Tracing 1)with threshold concentration and E_{max} 3×10^{-7}M and 3×10^{-5}M, respectively may be due to activation of available $\acute{a}_{1a/d}$ adrenoceptors (Kathirvel *et al.,* 2010). ACh-mediated relaxation of blood vessels is predominantly mediated by the production of NO in vascular endothelial cells. NO is generated by the endothelial enzyme NOS (Nitric oxide synthase) from the substrate L-arginine. ACh may have other effects on the endothelium, such as the release of prostaglandin H_2 (Kato *et al.,* 1990), endothelial-derived contracting factor, or endothelial-derived hyperpolarizing factor (Zanchi *et al.,* 1995). Vasodilation induced by ACh via mechanisms other than NOS activity is resistant to the blocking effect of L-NAME. In the absence of endothelium, ACh constricts the small resistance vessels (Richard *et al.,* 1991). It was observed that addition of acetylcholine (0.1-100 μM) to the perfusate containing the unrubbed rings elicited no significant relaxation of goat ruminal artery (see Table 1). The absence of a relaxing effect with acetylcholine was somewhat surprising since acetylcholine releases EDRF (endothelium derived relaxing factor) in most mammalian blood vessels but this kind result is in accordance with some of the earlier reports on human umbilical vessels (Van de Voorde *et al.,* 1987), bovine intrapulmonary veins (Ignarro *et al.,* 1987), canine basilar arteries (Kanamaru *et al.,* 1987) and goat ruminal artery

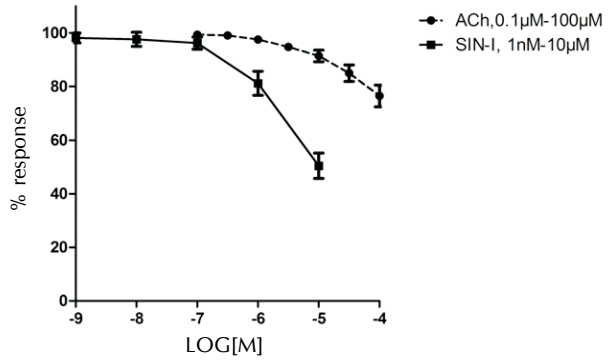


Figure 1: Effect of Ach (0.1μM-100μM) and SIN-I (1ςM-10μM) on endothelium intact goat ruminal artery

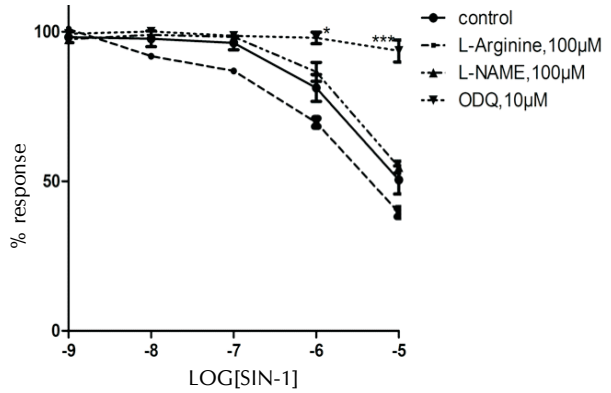
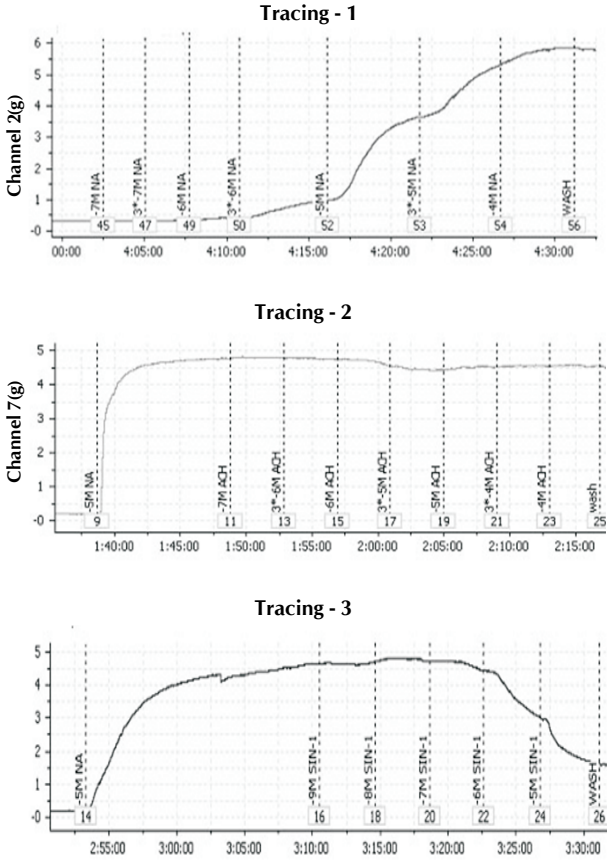


Figure 2: SIN-I (1ηM-10μM) induced relaxation on arterial rings precontracted with NA, 10μM in presence of L-Arginine, 100μM, L-NAME, 100μM and ODQ, 10μM.Control = NA, 10μM + SIN-1, 1ηM-10μM; * $p < 0.05$; *** $p < 0.001$

(Kathirvel and Parija, 2009). Although the exact explanation for this event cannot be given here but this effect is surely not due to an enzymatic destruction of acetylcholine because of a unremarkable but small relaxing effect (only 29%) (Table 1) observed during the study. However this result can be explained as due to lack of appropriate sensitive muscarinic receptors in the endothelial cells leading to EDRF release (Van de Voorde *et al.,* 1987).

In contrast to acetylcholine we observed a potent relaxation (61.69%) (See Table 1) of this artery in response to SIN-1(1ηM-10μM) which is an endothelium independent exogenous nitric oxide donor. In this artery, It was observed that the vascular response to SIN-1 ($P^D2 = 5.37 \pm 0.15$) was not affected by the NOS blocker L-NAME, 100μM ($P^D2' = 5.59 \pm 0.19$). In another set of experiment NOS activator L-arginine, 100μM did not augment the SIN-1 relaxation (Table 2) which confirmed our observations with L-NAME and strengthen our hypothesis that SIN-1 has some other mechanism of action other than activation of NOS in this artery. This finding is in corroboration with an earlier report in feline lower esophageal center (Jun *et al.,* 2003).

Nitric oxide (NO) donors such as nitroglycerin (NTG), Snitroso-N-acetylpenicillamine (SNAP) and linsidomine (SIN-1) are generally believed to elicit their vasodilatory effects through a common mediator, NO (Feelisch, 1993), that can bind to the heme site of soluble guanylatecyclase (sGC), activating the enzyme and catalyzing the conversion of GTP to cGMP (McDonald and Murad, 1995). Accumulated cellular cGMP then lowers intracellular calcium, leading to vasodilation (McDonald and Murad, 1996). Therefore, the vasodilatory action of NO donors is generally thought to occur primarily through the activation of the heme site of sGC. During this experiment, it was observed that SIN-1(1ηM-10μM) effect was totally blocked (maximal relaxation was 8.96% only) by addition of a more selective soluble guanylatecyclase inhibitor, ODQ,10μM which clearly indicated towards direct or indirect activation of s-guanylatecyclase by SIN-1 which was in accordance with the hypothesis that NO and NO donors mostly cause relaxation of vascular smooth muscle via cyclic guanosine 3′,5′-monophosphate (cGMP) accumulation (Moncada *et al.,* 1991). However this interpretation is just preliminary because NO /sGC/cGMP/ PKG pathway is not universal for all blood vessels. Activation of sGC may cause relaxation due to opening of K^+ channels (Bouchard *et al.,* 1994) or Ca^{2+} influx (Adachi *et al.,* 2002) and the NO donors generate an array of interrelated redox forms, nitrosoniumcation (NO^+), nitric oxide ($NO\cdot$) and nitroxyl anion (NO-), whose properties and reactivity are different (Stamler *et al.,* 1992) so warrants further work. Basing on our results, It



Tracing 1: NA (0.1-100μM) induced concentration dependent contractile response in endothelium intact goat ruminal artery rings; Tracing 2: Ach (0.1-100μM) induced relaxation; Tracing 3: SIN-1(1ηM-10μM) induced relaxation

was concluded that exogenous nitric oxide donor, SIN-1is a better relaxing agent than acetylcholine in goat ruminal artery and this effect is due to direct or indirect activation of sGC. However more detail study is needed to know the cellular mechanism.

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