European Journal of Pharmacology 609 (2009) 113–117

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular Pharmacology

L-arginine-induced dilatation of goat coronary artery involves activation of K_{ATP} channels

Dilip K. Deka [a,*], Santosh K. Mishra [b], Vellanki Raviprakash [b]

[a] Department of Pharmacology & Toxicology, AAU, Khanapara, Guwahati-781 022, India
[b] Division of Pharmacology, IVRI, Izatnagar, Bareilly-243122, India

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 26 December 2008
Accepted 1 March 2009
Available online 16 March 2009

Keywords:
Coronary artery
Vasodilatation
K_{ATP} channel
L-arginine

ABSTRACT

In the present study, the mechanism of relaxant response of nitric oxide precursor, L-arginine, was investigated in goat isolated coronary artery. L-arginine (1 mM) reversed the U-46619 (1 μM)-induced contraction both in endothelium-intact and endothelium-denuded arterial ring preparations. L-arginine analogues, L-NAME, L-NNA and L-NMMA and the guanylyl cyclase inhibitor, methylene blue failed to attenuate the relaxant response of L-arginine. These observations negate the involvement of nitric oxide in mediating the relaxation by L-arginine. K_{ATP} channel blocker, glibenclamide (3 μM), abolished the vasorelaxant responses of L-arginine in endothelium-denuded preparations, thereby suggesting the involvement of K_{ATP} channels. Further, L-arginine also failed to induce relaxation of the coronary arterial rings constricted with K^+ (80 mM)-PSS. Taken together, the results of the present study suggest that L- arginine relaxes goat isolated coronary artery through activation of K_{ATP} channels.

1. Introduction

Nitric oxide (NO) is known to account for the biological activity of endothelium-derived relaxing factor (EDRF) (Palmer et al., 1987; Ignarro et al., 1987) which plays a vital role in regulating vascular smooth muscle functions. It was shown that NO hyperpolarizes the rabbit mesenteric arteries through opening of K_{ATP} channels, with accumulation of cyclic GMP as an intermediate (Murphy and Brayden, 1995). It is interesting to note that inhibition of basal NO release was shown to augment the vasodilator responses of potassium channel openers in rabbit-ear artery (Randall et al., 1994) and in rat isolated superior mesenteric arterial bed (McCulloch and Randall, 1996). On the contrary, exogenous NO donor mediated vasodilatations in goat coronary artery is independent of K_{ATP} channel activation (Deka et al., 1997).

K^+ channels are critical in integrating the dilator and constrictor actions of a variety of vasoactive substances through the regulation of membrane potential of vascular smooth muscle (Nelson and Quayle, 1995). The nitric oxide synthase inhibitors, L-arginine analogues like L-NNA and L-NMMA are shown to inhibit ATP-sensitive K^+ channels in pial arteriolar smooth muscle, an effect that could be reversed by L-arginine, an essential amino acid (Kontos and Wei, 1996). Although there is little information on L-arginine directly activating K^+ channels to produce vasodilatation, patch clamp and tension studies provide evidences that L-arginine activates K_{ATP} channels in porcine coronary smooth muscle cells (Miyoshi et al., 1994) or relaxes rat aorta by activating K_{Ca} channels (Hall et al., 1996).

L-arginine has been reported to have beneficial circulatory effects in patients with essential and secondary hypertension (Nakaki et al., 1990; Nakaki and Kato, 1994). Since the discovery of L-arginine as a precursor of endothelium-derived relaxing factor (EDRF) or NO (Palmer et al., 1988; Sakuma et al., 1988), it is generally believed that the vascular effects of the amino acid are mediated by NO which is either derived from the endothelial cells or synthesized by the vascular smooth muscle cells upon stimulation by inducible nitric oxide synthase (Moncada et al., 1991). Exogenous administration of the amino acid results in a marked decrease in the blood pressure in these patients (Hishikawa et al., 1992, 1993). However, both *in vivo* (Calver et al., 1990) and *in vitro* (Thomas et al., 1989) studies have shown that L-arginine mediated vasodilatation is independent of NO production. Nevertheless, the mechanism of vasodilator action of L-arginine remains to be clarified.

It is well known that the opening of K^+ channels is an efficient mechanism leading to vasodilatation. Precisely, the activation of K^+ channels causes membrane potential hyper-polarization and a consequential decrease in the open probability of voltage-dependent calcium channels leading to vasorelaxation (Meisheri et al., 1990). Both K_{ATP} (Deka et al., 1997) and K_{Ca} (Deka et al., 2005) channels are present in goat coronary artery. Keeping these facts in mind we wanted to examine: 1) whether, L-arginine could relax goat isolated coronary artery and 2) if so, whether K^+ channels are involved in the mechanism of arterial relaxation by the amino acid.

* Corresponding author.
E-mail address: drdilipdeka@rediffmail.com (D.K. Deka).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.03.031

2. Materials and methods

2.1. Isolated tissue experiments

Goat hearts were collected from a local abattoir and were carried to the laboratory in oxygenated (95% $O_2 + 5\%$ CO_2), cold physiological saline solution (PSS) of the following composition mM: NaCl, 118; KCl, 4.7; $CaCl_2$. $2H_2O$, 2.5; $MgSO_4$. $7H_2O$, 1.2; $NaHCO_3$, 11.9; KH_2PO_4, 1.2 and glucose 11.1. The left anterior descending or circumflex coronary artery was isolated within 30 min and was cleaned of fat and connective tissue. The coronary tissue was then cut into 4–5 rings of about 3 mm length and 1.5–2 mm external diameter. In most of the rings the endothelium was removed mechanically by gently rubbing the intimal surface of the arterial segments with a fine forceps. The arterial rings with or without endothelium were individually suspended between two stainless steel angular hooks and mounted in an organ bath containing 20 ml PSS and continuously bubbled with carbogen (95% $O_2 + 5\%$ CO_2) at 37° ± 0.5 °C (pH 7.4). The resting force was adjusted to 1.5 g and the tissues were allowed to equilibrate for a period of 90 min during which the PSS was changed every 15 min. Following equilibration, the tissues were subjected to sub-maximal contraction using U-46619 (1 μM). At the plateau of contraction, L-arginine (1 mM) was added. Isometric force was recorded in an ink writing polygraph using a force transducer (Recorders and Medicare, India). The absence of functional epithelium was verified by application of alpha$_2$-adrenoceptor agonist clonidine which fails to relax goat coronary artery in the absence of endothelium.

2.2. Experimental protocol

2.2.1. Characteristics of L-arginine induced relaxation in endothelium-intact and endothelium-denuded preparations

Coronary artery rings with or without endothelium were constricted with U-46619 (1 μM) and when the contractions reached a steady state (after 15–20 min), L-arginine (1 mM) was added to produce relaxation. In order to eliminate the interference by vasoactive substances released from the endothelium, experiments were done on endothelium-denuded preparations. To examine the stereo specificity of the relaxant response of L-arginine, we used both D- and L-arginine in endothelium-denuded preparations.

2.2.2. Effect of L-arginine analogues and methylene blue on the relaxant responses of L-arginine in endothelium-denuded coronary arteries

L-arginine analogues like L-NAME, L-NNA and L-NMMA are known to inhibit both cNOS and iNOS and thereby, decrease the synthesis of NO (Moncada and Higgs, 1995). In addition, some of these analogues like L-NNA and L-NMMA have been shown to antagonise the vascular K_{ATP} channels directly (Kontos and Wei, 1996). Therefore, we wanted to examine if the vasoactive action of L-arginine could be blocked by L-arginine analogues. After an equilibration period of 90 min, control relaxation response to L-arginine was elicited in tissues contracted with U-46619 (1 μM). The tissues were then washed several times with PSS and then incubated with either L-NAME (100 μM), L-NNA (100 μM) or L-NMMA (100 μM) for 45 min. Thereafter, the tissues were again subjected to sub-maximal contraction with U-46619. At the plateau of contraction, L-arginine was added to the bath to see any interference by the L-arginine analogues in L-arginine induced relaxation.

Similarly, guanylyl cyclase inhibitor, MB (3 μM) was used to study the contribution of cyclic GMP in the relaxant responses of L-arginine.

2.2.3. Role of K^+ channels in the mechanism of vasodilatation by L-arginine

In order to examine the role of K^+ channels on the relaxant responses of L-arginine in goat coronary artery (endothelium-denuded), relaxation with L-arginine was elicited either in the absence or presence of K_{Ca} channel blocker TEA (1 mM) or K_{ATP} channel blocker glibenclamide (3 μM) in U-46619 constricted coronary arterial rings. The tissues were then washed several times and incubated with either TEA or Glibenclamide for 45 min. The coronary arterial rings were again subjected to sub-maximal contraction with U-46619 followed by application of L-arginine at the plateau of contraction.

2.2.4. Effect of high K^+ (80 mM) saline solution on responses to L-arginine

High K^+ (80 mM), that attenuates the K^+ gradient across the cell membrane, is one of the experimental protocols to assess the involvement of K^+ channels in the mechanism of action of a vasodilator (Meisheri et al., 1990). In the present set of experiments, coronary artery ring segments were contracted with K^+ (80 mM) solution (prepared by equimolar replacement of Na^+ in the PSS) and at the plateau of contraction, L-arginine (1 mM) was added to elicit relaxation. The results were compared with coronary artery rings constricted with U-46619 and relaxed with L-arginine.

2.3. Drug solution

Both D-arginine hydrochloride (Sigma) and L-arginine hydrochloride (Sigma) were prepared as stock solutions of 100 mM in PSS. L-NAME (N^G-nitro-L-arginine methyl ester), L-NNA (N^G-nitro-L-arginine) and L-NMMA (N^G-monomethyl-L-arginine) (all from Sigma) were prepared as 10 mM solution in distilled water. Methylene blue (MB) (Sigma) was prepared as a stock solution of 10 mM in distilled water. Pinacidil (gift from Leo Pharmaceuticals, USA) was prepared as a stock solution of 10 mM in 0.1 N HCl. TEA (Tetraethyl ammonium) (E. Merck, India) was prepared as a stock solution of 100 mM in distilled water. Stock solutions (10 mM) of glibenclamide (gift from Hoechst) and U-46619 (9, 11-dideoxy-9α, 11α-methanorepoxy PG $F_{2\alpha}$) (gift from Clayman Chemicals, USA) were prepared in dimethylsulphoxide.

2.4. Statistics

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

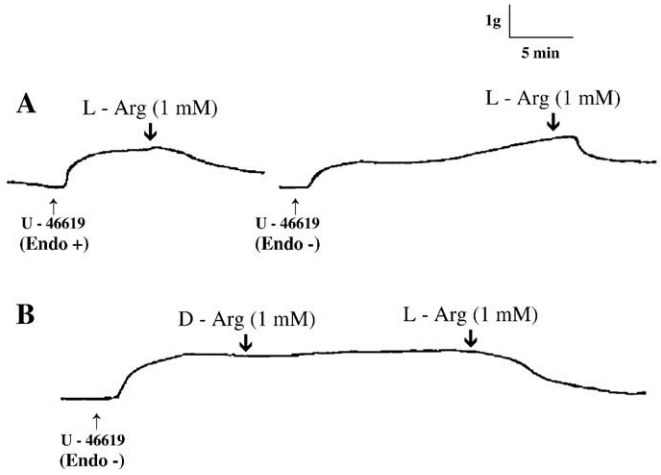

Fig. 1. A) Original traces showing the relaxant responses of L-arginine (1 mM) in endothelium-intact (endo+) and denuded (endo-) goat isolated coronary arterial rings constricted with U-46619 (1 μM). B) Original traces showing lack of relaxant effect of D-arginine (1 mM) in endothelium-denuded goat coronary artery rings constricted with U-46619 (1 μM) and the vasodilator response of L-arginine (1 mM) in the continued presence of D-arginine.

3. Results

3.1. Characteristics of L-arginine responses in goat isolated coronary artery

Fig. 1A depicts the characteristics of the relaxant responses of L-arginine (1 mM) in coronary artery ring segments constricted with U-46619 (1 μM). L-arginine caused reversal of the contracture induced by U-46619 both in endothelium-intact and endothelium-denuded preparations (Fig. 1A). The absolute forces of contraction induced with U-46619 were 0.73 ± 0.09 g ($n = 3$) in endothelium-intact and 0.86 ± 0.03 g ($n = 36$, pooled data) in endothelium-denuded coronary arterial rings. The percent relaxations produced by L-arginine were 72.67 ± 7.97% ($n = 3$) and 68.69 ± 1.69% ($n = 36$, pooled data) in endothelium-intact and endothelium-denuded coronary artery rings, respectively. However, experiments with L-arginine were conducted in endothelium-denuded preparations in the present study so as to eliminate the interference by the vasoactive substances that may be basally released from the vascular endothelium. Fig. 1B depicts that D-arginine (1 mM) could not produce relaxation of the isolated coronary arterial rings of goat constricted with U-46619 (1 μM).

3.2. Effects of L-arginine analogues and methylene blue on the responses to L-arginine

Fig. 2 summarises the effects of L-arginine analogues and guanylyl cyclase inhibitor methylene blue on the relaxant responses of L-arginine (1 mM) in endothelium-denuded preparations. Pre-treatment of the tissues with L-NAME (100 μM) for 45 min had no significant effect on the contractions induced by U-46619 (1 μM) and thus, the absolute force generated by U-46619 in the absence and presence of L-NAME was 0.86 ± 0.03 g ($n = 36$) and 0.88 ± 0.06 g ($n = 6$), respectively. However, the NO synthase inhibitor had significantly augmented ($p < 0.05$) the relaxant response induced by L-arginine in U-46619-constricted arterial ring segments (Fig. 3A). Hence, the percent relaxations in absence and presence of L-NAME were 68.69 ± 1.69 ($n = 36$) and 83.0 ± 5.13 ($n = 6$), respectively.

Fig. 3B depicts the effect of L-NNA (100 μM) on the relaxant response of L-arginine in arterial rings constricted with U-46619. Pre-treatment of the arterial rings with L-NNA for 45 min had no effect on the amplitudes of contraction and the absolute force was 0.86 ± 0.03 g ($n = 36$) in control as against 0.88 ± 0.03 ($n = 6$) g in L-NNA-treated tissues. However, L-NNA augmented ($p < 0.05$) the relaxant responses of L-arginine on coronary artery rings contracted with U-46619. The percent relaxations in L-NNA-treated arterial rings was 77.01 ± 2.82%, ($n = 6$) as against 68.69 ± 1.69% ($n = 36$) in the control tissues.

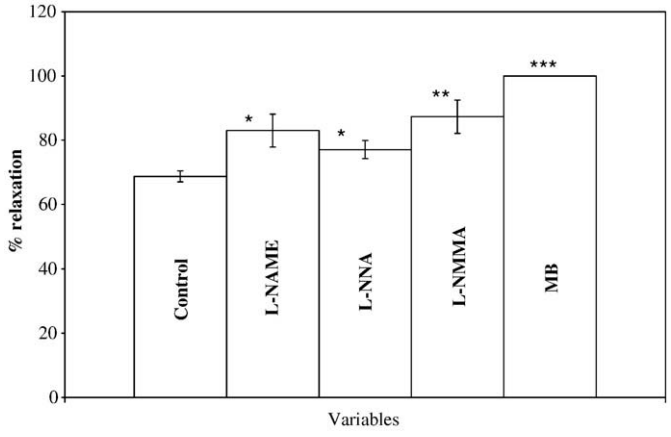

Fig. 2. Bar diagram showing percent relaxations produced by L-arginine in endothelium-denuded goat coronary arterial rings constricted with U-46619 in absence and in presence of L-NAME, L-NNA, L-NMMA and methylene blue. Values represent mean ± SEM; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

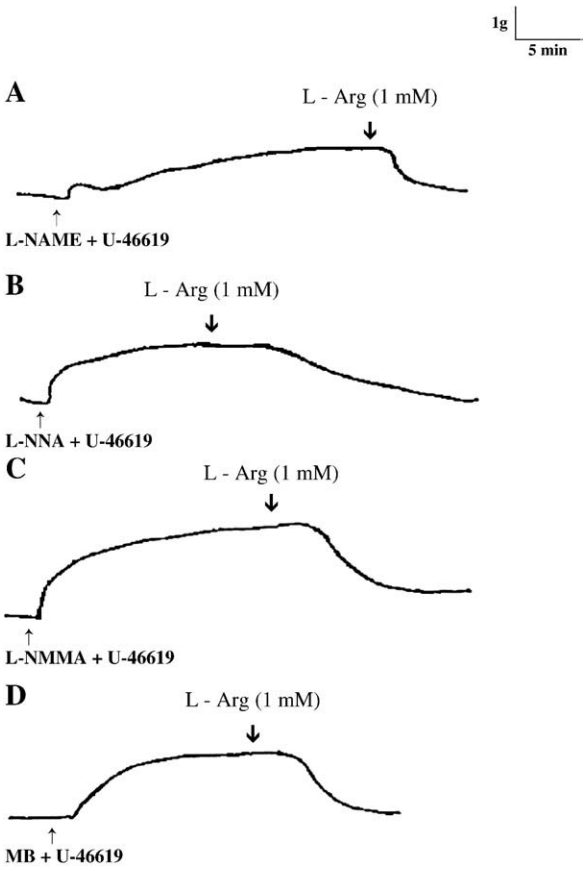

Fig. 3. Original traces showing the effects of L-arginine analogues and guanylyl cyclase inhibitor MB on the relaxant responses of L-arginine (1 mM) in endothelium-denuded goat isolated coronary artery rings contracted with U-46619 (1 μM). Pre-treatment of the arterial rings for 45 min with L-NAME (0.1 mM). (A), L-NNA (0.1 mM). (B) and L-NMMA (0.1 mM). (C) had significantly augmented the relaxant responses of L-arginine. Similarly, pre-treatment with methylene blue (3 μM) for 45 min also augmented the relaxant response of L-arginine achieving 100% relaxation (D).

Fig. 3C illustrates the effect of another NO synthase inhibitor, L-NMMA (100 μM) on the relaxant responses of L-arginine in coronary artery rings constricted with U-46619. Pre-treatment of the tissues with L-NMMA for 45 min significantly augmented ($p < 0.001$) the contractions elicited by U-46619. Thus, the mean absolute force in presence of L-NMMA was 1.48 ± 0.11 g ($n = 6$) as against 0.86 ± 0.03 g ($n = 36$) in the controls. Interestingly, L-NMMA also significantly augmented ($p < 0.01$) the relaxant responses of L-arginine in U-46619-constricted arteries and the amplitudes of relaxation in absence and presence of NO synthase inhibitor were 68.69 ± 1.69% ($n = 36$) and 87.3 ± 5.19% ($n = 6$), respectively.

Fig. 3D shows the effect of methylene blue (10 μM) on the vasorelaxant responses of L-arginine (1 mM) in goat isolated coronary artery. Pre-treatment of the tissues with MB for 45 min significantly augmented ($p < 0.001$) the contractions caused by U-46619(1 μM). Thus, the absolute forces generated in absence and presence of MB were 0.86 ± 0.03 g ($n = 36$) and 1.03 ± 0.02 g ($n = 6$), respectively. The vital blue, at this concentration, also augmented ($p < 0.001$) the relaxation elicited by L-arginine in coronary artery rings constricted with U-46619. Thus, 100% relaxation ($n = 6$) was obtained in tissues pre-treated with MB as compared to 68.69 ± 1.69% ($n = 36$) in the controls.

3.3. Effects of K^+ channel blockers on the relaxant responses of L-arginine and pinacidil in goat coronary artery

All the experiments with K^+ channel blockers were conducted in endothelium-denuded preparations. Fig. 4 demonstrates the effects of pre-treatment of coronary artery rings with K^+ channel blockers, TEA (1 mM) and glibenclamide (3 μM) for 45 min and constricted with U-46619 on the relaxant responses of L-arginine.

Tissues pre-treated with K_{Ca} channel blocker, TEA for 45 min had augmented ($p<0.001$) on the contraction evoked by U-46619 (Fig. 4A). Thus, the amplitudes of contraction in absence and in presence of TEA were 0.86 ± 0.03 g ($n=36$) and 1.32 ± 0.09 g ($n=6$), respectively. TEA also augmented ($p<0.001$) the relaxant responses of L-arginine in U-46619-contracted arteries. Hence, the relaxations elicited with L-arginine in the control and in the TEA-treated tissues were $68.69 \pm 1.69\%$ ($n=36$) and $91.16 \pm 3.1\%$ ($n=6$), respectively.

Interestingly, the K_{ATP} channel blocker, glibenclamide (3 μM) had also augmented ($p<0.05$) the contractions induced by U-46619 in goat coronary artery preparations. Thus, the absolute forces of contractions were 0.86 ± 0.03 g ($n=36$) and 1.07 ± 0.09 g ($n=6$) in absence and in presence of glibenclamide, respectively. Further, L-arginine (1 mM) failed to relax the tissues when pre-treated with glibenclamide for 45 min (Fig. 4B). Fig. 4C shows that K_{ATP} channel opener pinacidil (3 μM) which produced $75 \pm 2.89\%$ ($n=3$) relaxation in control tissues failed to relax the tissues when pre-treated with glibenclamide (3 μM) for 45 min.

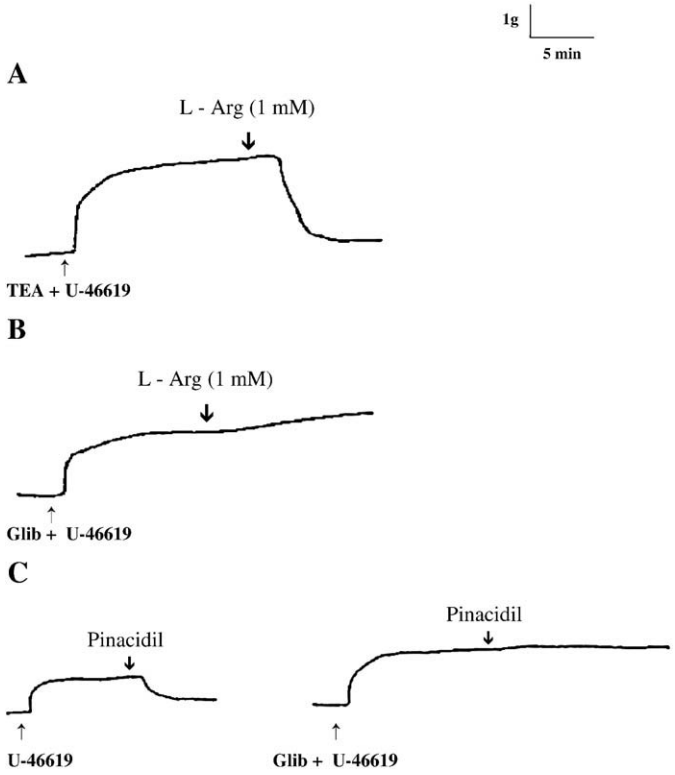

Fig. 4. Original traces showing the effect of pre-treatment of coronary artery rings with TEA (1 mM) and Glibenclamide (3 μM) on the relaxant response of L-arginine (1 mM). Pre-treatment with K_{Ca} channel blocker TEA significantly augmented ($p<0.05$) the relaxation induced by L-arginine (A). On the other hand, K_{ATP} channel blocker glibenclamide inhibited the relaxant response of L-arginine (B). (C) depicts comparative relaxant effect of K_{ATP} channel opener Pinacidil and its blockade with glibenclamide (3 μM) on endothelium-denuded goat isolated coronary artery rings contracted with U-46619.

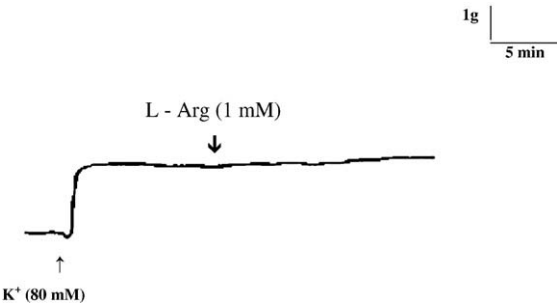

Fig. 5. Original traces showing contractile response induced with K^+ (80 mM) PSS in endothelium-denuded goat isolated coronary rings and the effect of L-arginine (1 mM) added at the plateau of contraction. Note that L-arginine failed to relax the tissues when constricted with high K^+ PSS (80 mM).

3.4. Influence of high extra cellular K^+ on the relaxant responses of L-arginine

Fig. 5 illustrates the amplitudes of L-arginine (1 mM)-induced relaxation in coronary artery rings (endothelium-denuded) constricted with either U-46619 (1 μM) or K^+ (80 mM) ($n=2$). As shown in the figure, L-arginine could relax the coronary arterial rings when constricted with U-46619, but failed to do so when constricted with K^+ (80 mM).

4. Discussion

The results of the present study suggest that L-arginine-induced relaxation of goat coronary artery is mediated by K_{ATP} channels and there appears to be no role for NO in this mechanism. These conclusions are based on the following observations-

1. L-arginine elicited relaxant response in goat coronary artery rings constricted with U-46619. Pinacidil, a known K_{ATP} channel opener, also elicited relaxation in goat coronary artery rings constricted with U-46619.
2. K_{ATP} channel blocker, glibenclamide was able to inhibit the vasodilator response of both L-arginine and pinacidil.
3. Neither NOS inhibitor L-NAME, L-NMMA or L-NNA nor guanylyl cyclase inhibitor methylene blue could inhibit the vasodilator response of L-arginine.
4. In comparison to the relaxant response achieved with L-arginine in coronary arterial rings constricted with U-46619, the vasodilator response to L-arginine was not observed when the tissues were contracted with high K^+ (80 mM) PSS, which is known to abolish the K^+ gradient across the cell membrane.

It is of interest to note that Miyoshi et al. (1994) found that L-arginine opened K_{ATP} channels in single smooth muscle cells isolated from porcine coronary artery preparation. The activation of the channel was inhibited by pre-treatment with L-arginine analogues and therefore, it was concluded that L-arginine-mediated K_{ATP} channel activation was due to generation of NO by the essential amino acid. In the present study, however, we found no evidence for an intermediate role of NO in the activation of K_{ATP} channels by L-arginine. This is supported by the findings that not only L-arginine analogues failed to attenuate the vasodilator responses of L-arginine, but the guanylyl cyclase inhibitor, methylene blue also did not antagonise the responses of L-arginine. Instead, methylene blue was found to augment the relaxant responses of L-arginine. Methylene blue is known to depolarize vascular smooth muscle, which is unrelated to its effect on the NO mechanism (Murphy and Brayden, 1995). It is, therefore, possible that L-arginine is more potent in presence of methylene blue in relaxing the goat coronary artery smooth muscle through a greater opening of the K_{ATP} channels from a depolarized state. The role of NO in the activation of K_{ATP} channel in goat coronary

artery is further ruled out by the fact that the K_{ATP} channels did not mediate relaxations of goat coronary artery by NO donors SIN-1 and SNP (Deka et al., 1997). Since, NOS-inhibitors as well as guanylyl cyclase inhibitor MB do not antagonize the effect of L-arginine, there fore, L-arginine besides being a substrate for NO synthesis, can directly dilate goat coronary vessels through activation of K_{ATP} channels which is of significant importance. However, our study cannot explain why L-arginine analogues augmented the L-arginine-induced vasorelaxation in goat coronary artery.

The K_{ATP} channel blocker, glibenclamide abolished the vasorelaxant responses of L-arginine as well as that of pinacidil, a known K_{ATP} channel activator. This observation, thus, suggest that L-arginine relaxes goat coronary artery through activation of K_{ATP} channels. In an earlier study, glibenclamide was shown to inhibit the induction of iNOS in cultured macrophages (Wu et al., 1995). However, this particular mechanism may not be involved in the attenuation of the vasorelaxant responses of L-arginine in goat coronary artery by glibenclamide. This is evident from the fact that L-arginine analogues such as L-NAME, L-NNA and L-NMMA, which are non-specific inhibitors of iNOS failed to attenuate L-arginine-induced relaxation of goat coronary artery. Taken together these results provide the first direct evidence that K_{ATP} channels are involved in the vasodilator responses of L-arginine.

Hall et al. (1996) have shown that L-arginine caused large relaxations in rat isolated aorta with bacterial lipopolysaccharide that were markedly inhibited by TEA. Therefore, they suggested a role for the involvement of Ca^{2+}-activated K^+ channels in the mechanism of NO-dependent vasodilator action of L-arginine in rat aorta. In the present investigation however, we observed that the K_{Ca} channel blocker TEA markedly augmented the relaxant response of L-arginine. If L-arginine is increasing the conductance through K_{ATP} channels it is expected that TEA would augment the vasorelaxant responses of the amino acid. This is because, K^+ channel blockade with TEA may depolarise the arterial cell membrane and thereby leading to an increase in the driving force of an outward K^+ current. Murphy and Brayden (1995) have shown that K^+ channel blockers like Ba^{2+}, TEA and 4-AP increase the magnitude of hyperpolarisation to K_{ATP} channel opener, Cromakalim in rabbit mesenteric artery.

If L-arginine-induced relaxation of goat coronary arterial smooth muscle results from an increase in K^+ conductance through K_{ATP} channel as suggested from the present study, then, they could be attenuated when the K^+ gradient across the cell membrane is reduced. We observed that L-arginine relaxes the tissue when constricted with U-46619. However, the amino acid failed to relax the arterial smooth muscle constricted with high K^+ (80 mM) saline solution. It is well known that when the extra cellular K^+ is high, the E_K is close to the resting membrane potential. Therefore, any drug that activates the opening of K^+ channel would fail to change the net K^+ efflux and thus, would fail to hyperpolarize and dilate the vascular smooth muscle (Weir and Weston, 1986).

In conclusion, the findings of the present study suggest that goat coronary artery vasodilation due to L-arginine is mediated through activation of K_{ATP} channels. At the same time our study also rules out the involvement of nitric oxide in the activation of K_{ATP} channels in the L-arginine-induced vasodilation in goat coronary artery.

References

Calver, A., Collier, J., Vallance, P., 1990. Nitric oxide and cardiovascular control. Exp Physiol. 78, 303–326.

Deka, D.K., Raviprakash, V., Mishra, S.K., 1997. K_{ATP} channels do not mediate vasodilation by 3-morpholinosydnonimines in goat coronary artery. Eur. J. Pharmacol. 157–164 330/2–3.

Deka, D.K., Raviprakash, V., Mishra, S.K., 2005. Sodium nitroprusside relaxes goat coronary artery through activation of calcium-dependent K^+ channels. Ind. J. Exp. Biol. 43, 324–329.

Hall, S., Turcato, S., Clapp, L., 1996. Abnormal activation of K^+ channels underlies relaxation to bacterial lipopolysaccharides in rat aorta. Biochem. Biophys. Res. Commun. 224, 184–190.

Hishikawa, K., Nakaki, T., Suzuki, H., Kato, R., Saruta, T., 1992. L-arginine as an antihypertensive agent. J Cardiovasc. Pharmacol. 20 (Suppl. 12), S196–S197.

Hishikawa, K., Nakaki, T., Suzuki, H., Kato, R., Saruta, T., 1993. Role of L-arginine nitric oxide pathway in hypertension. J. Hypertens. 11 (6), 639–645.

Ignarro, L.J., Burga, G.M., Wood, K.S., Byrns, R.E., Choudhury, G., 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Natl. Acad. Sci. U. S. A. 84, 926.

Kontos, H.A., Wei, E.P., 1996. Arginine analogues inhibit responses mediated by ATP-sensitive K^+ channels. Am. J. Physiol. 271, H1498–H1506.

McCulloch, A.I., Randall, M.D., 1996. Modulation of vasorelaxant responses to potassium channel openers by basal nitric oxide in the rat isolated superior mesenteric arterial bed. Br. J. Pharmacol. 117, 859–866.

Meisheri, K.D., Cipkus Dubray, L.A., Oleynek, J.J., 1990. A sensitive *in vitro* functional assay to detect K^+ channel-dependent vasodilators. J. Pharmacol. Methods 24, 251.

Miyoshi, H., Nakaya, Y., Moritoki, H., 1994. Non-endothelial derived nitric oxide activates the ATP-sensitive K^+ channels of vascular smooth muscle cells. FEBS lett. 345, 47–49.

Moncada, S., Higgs, E.A., 1995. Molecular mechanism and therapeutic strategies related to nitric oxide. FASEB J. 9, 1319–1330.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, patho-physiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Murphy, M.E., Brayden, J.E., 1995. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. J. Physiol. (Lond) 486, 47–58.

Nakaki, T., Kato, R., 1994. Beneficial circulatory effects of L-arginine. Jpn. J. Pharmacol. 66, 167–171.

Nakaki, T., Hishikawa, K., Suzuki, H., Saruta, T., Kato, R., 1990. L-arginine-induced hypotension. Lancet 336 (8716), 696.

Nelson, M.T., Quayle, J.M., 1995. Pharmacological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822 (Cell Physiol. 37).

Palmer, R.M.J., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for biological activity of endothelium-derived relaxing factor. Nature (London). 327, 524.

Palmer, R.M.J., Rees, D.D., Ashton, D.S., Moncada, S., 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Randall, M.D., Ujiie, H., Griffith, T.M., 1994. Modulation of vasodilatation to levcromakalim by adenosine analogues in the rabbit ear: an explanation for hypoxic augmentation. Br. J. Pharmacol. 112, 49–54.

Sakuma, I., Stuchr, D.J., Cross, S.S., Nathan, C., Levi, R., 1988. Identification of arginine as a precursor of endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. U. S. A. 85, 8664–8667.

Thomas, G., Hecker, M., Ramwell, P.W., 1989. Vascular activity of polycations and basic amino acids-L-arginine does not specifically elicit endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 158, 177–180.

Wu, C.-C., Thiemermann, C., Vane, J.R., 1995. Glibenclamide-induced inhibition of the expression of inducible nitric oxide synthase in cultured macrophages and in the anaesthetised rat. Br. J. Pharmacol. 114, 1273–1281.

Weir, S.W., Weston, A.H., 1986. The effects of BRL 34915 and nicorandil on electrical and mechanical activity and on ^{86}Rb efflux in rat blood vessels. Br. J. Pharmacol. 88, 121–128.