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VASCULAR ACTIVITY OF POLYCATIONS AND BASIC AMINO ACIDS: L-ARGININE DOES NOT SPECIFICALLY ELICIT ENDOTHELIUM-DEPENDENT RELAXATION

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Irrespective of their stereochemistry (D- or L-form), polycations such as poly-lysine, poly-arginine and poly-histidine elicited endothelium dependent relaxation of pre-contracted rat aortic rings in a dose-dependent manner ($ED_{50} \leq 10^{-7}$ M). In contrast, the basic amino acids arginine, glutamine, histidine and lysine caused only endothelium-potentiated relaxation at high concentrations ($ED_{50} > 10^{-3}$ M). Both heparin (1U/ml) and dextran sulphate (10 μg/ml) abolished relaxation by the polycations but had no effect on the responses to the basic amino acids or acetylcholine. These results indicate that the vasodilatory property of the polycations is due to an electrostatic interaction with anionic domains on the endothelial surface, whereas the basic amino acids elicit a non-specific relaxation. Therefore, L-arginine *per se* cannot be the immediate precursor of nitric oxide, the proposed endothelium-derived relaxing factor. © 1989 Academic Press, Inc.

Several vasoactive peptides which elicit endothelium-dependent relaxation contain the amino acid arginine in their sequence (1). Endothelium-dependent relaxation is associated with an increase in cyclic GMP in the underlying smooth muscle. Deguchi and Yoshioka reported that L-arginine and peptides which contain L-arginine activate soluble guanylate cyclase in neuroblastoma cells (2). We have previously shown that polycations like poly-L-arginine or poly-L-lysine induce endothelium-dependent relaxation at submicromolar concentrations and that this effect is inhibited by the guanylate cyclase inhibitor methylene blue (1). In contrast, L-arginine showed no significant vasoactive properties even up to millimolar concentrations, whereas arginine derivatives, such as L-arginine ethyl ester or N-α-benzoyl-L-arginine ethyl ester (BAEE) induce relaxation of pre-contracted rat aortic rings which is potentiated by the presence of endothelium (3).

Recent reports assert that the pharmacological properties of the endothelium-derived relaxing factor (EDRF) and nitric oxide (NO) are identical and that endothelial cells release NO when challenged with bradykinin (4,5). Moreover, it has been suggested that the guanidino group of L-arginine is the endogenous precursor of the NO molecule (6,7). Herein, we demonstrate that arginine can induce relaxation in rat aorta which is potentiated by the presence of endothelium, but (i) that this effect is only achieved at very high doses ($\geq 10^{-3}$ M), (ii) that it is not specific for the L-isomer, and (iii) that other basic amino acids possess similar properties. In addition, we studied the interaction between various polycations and anionic domains on the endothelial surface and compared the effect of charge neutralization on the relaxation responses to acetylcholine, the polycations and basic amino acids.

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MATERIALS AND METHODS

Poly-arginine and poly-lysine (D- and L-form, MW 40,000-100,000), D- and L-arginine, L-glutamine, L-lysine, L-lysine hydrochloride, L-histidine, heparin, dextran sulphate (MW 100,000), phenylephrine hydrochloride, acetylcholine chloride, methylene blue, and BAEE were purchased from Sigma Chemical Co (St. Louis, MO). Aortic rings were prepared from the thoracic aorta of male Sprague-Dawley rats and the relaxation studies were conducted as previously described (1).

RESULTS

The effects of poly-D-arginine and poly-L-arginine (MW 40,000) and poly-D-lysine and poly-L-lysine (MW 100,000) on rat aortic rings with and without endothelium are depicted in Fig. 1. Only the vessels with intact endothelium showed a dose-dependent relaxation which was reversed by addition of methylene blue but was not affected by the cyclooxygenase inhibitor indomethacin (Fig. 1).

Negatively charged molecules, such as heparin and dextran sulphate completely inhibited relaxation by poly-L-arginine, whereas they had no effect on either BAEE or acetylcholine induced relaxation (Fig. 2). Similar inhibitory effects were obtained for the other polycations (not shown). Fig. 3 shows the relaxation of intact aortic rings by D-and L-arginine, L-histidine and L-glutamine which can be also reversed with methylene blue. Moreover, we found that, independent of the presence of an intact endothelium, all the basic amino acids were effective and that, except L-lysine which elicited slight contraction when the base form was used, relaxation was observed irrespective whether the hydrochloride salt or the base form of the amino acids was used (data not shown).

The effect of equimolar concentration of D- and L-arginine on aortic segments with and without endothelium is shown in Table 1 which indicates that rings with endothelium exhibited enhanced relaxation with both isomers.

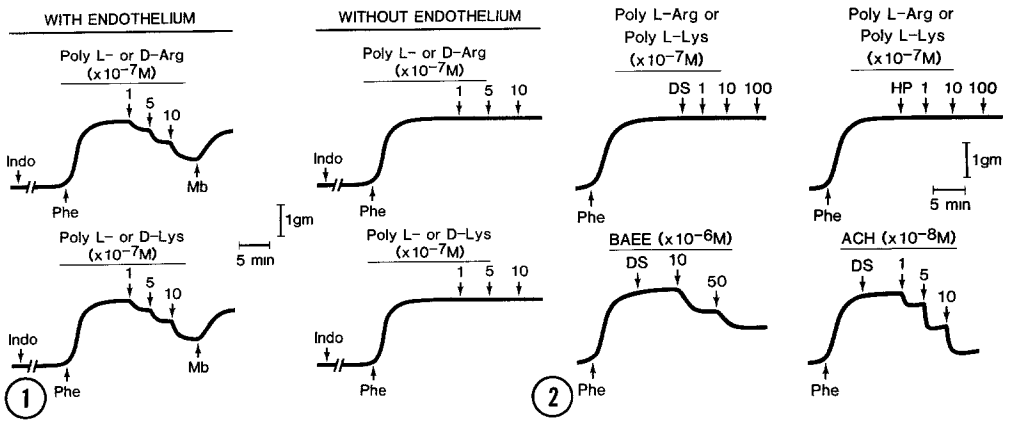

Fig. 1: *Representative tracings showing the effect of poly-L or D-arginine and poly-L or D-lysine on rings from rat thoracic aorta with and without endothelium.* The vessels were preconstricted with phenylephrine (Phe, 1 x 10^{-7} M) in the presence of indomethacin (Indo, 1 x 10^{-5} M) prior to addition of the polycations at the concentrations indicated. The relaxation was reversed by addition of methylene blue (Mb, 1 x 10^{-5} M).

Fig. 2: *Representative tracings showing the effect of charge neutralization on the relaxation response to the polycations, BAEE and acetylcholine.* Pre-contracted rat aortic rings (Phe, 1 x 10^{-7} M) were incubated with either heparin (HP, 1 U/ml) or dextran sulphate (DS, 10 μg/ml) for 3 min in the bath before addition of the polycations, BAEE or acethylcholine (ACH) at the concentrations indicated.

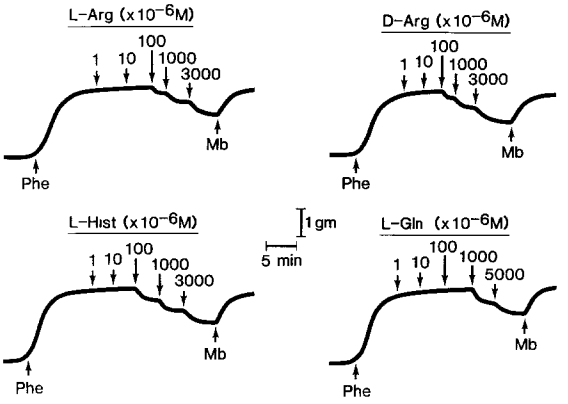

Fig. 3: *Representative tracings showing the effect of basic amino acids on rat aortic rings with intact endothelium.* The vessels were pre-constricted with phenylephrine (Phe, 1 x10^{-7} M) followed by the addition of cumulative doses of either L-arginine, D-arginine, L-histidine or L-glutamine. With each amino acid the relaxation response was reversed by methylene blue (Mb, 1 x 10^{-5} M).

DISCUSSION

We have previously reported that poly-L-arginine and poly-L-lysine elicit endothelium-dependent relaxation (1). Herein we confirm and extend these data by showing that the D-isomers of these polycations have the same effect and that this also applies for other polycations, such as poly-L-histidine (not shown). The inhibitory action of negatively charged molecules like heparin and dextran sulphate indicate an electrostatic interaction of the polycations with anionic sites present in the luminal surface of the endothelium that may trigger the release of EDRF. The presence of such anionic microdomains in capillary endothelium has been shown (8) and an interaction with polycations leads to rapid clustering of these anionic sites (9). Furthermore, the present studies show that vasodilation elicited by compounds such as acetylcholine, BAEE or the basic amino acids is not mediated by an interaction with these anionic sites, since here heparin and dextran sulphate were not effective. Interestingly, long term exposure of the aortic rings to the polycations caused a strong desensitization to both contractile and relaxing agonists, an effect which was not observed with acetylcholine or the arginine analogue (not depicted here). This effect may be due to cell injury and release of LDH as shown by Needham et al (10).

Table 1

Effect of equimolar concentrations (6.5 x 10^{-3} M) of L- and D-arginine on intact and denuded rat aortic rings

	% Relaxation*	
	with endothelium	without endothelium
L-Arginine	46.3 ± 8.9 (n = 4)	26.7 ± 5.8 (n = 4)
D-Arginine	40.0 ± 11.2 (n = 4)	15.0 ± 4.9 n = 4)

*Relaxation is expressed as percent of the pre-contraction induced by phenylephrine (1 x 10^{-7} M). Results are mean ± SEM.

Comparative pharmacological and chemical studies indicate that EDRF is NO (5). However, they cannot exclude that EDRF may be a closely related chemical entity or even a NO-carrier molecule like streptozotocin, a powerful vasodilator of rat aorta (Thomas, G. and Ramwell, P.W. (1988) *Eur. J. Pharmacol.*, submitted). The formation of NO from L-arginine has been reported in murine macrophages (6) and a recent study using isolated rabbit aorta showed that L-arginine generates NO from the endothelium (11). However, so far no attempt has been made to correlate the amount of NO derived from L-arginine with the degree of relaxation. In our hands even millimolar concentrations of L-arginine induce only weak vascular relaxation, whereas L-arginine containing peptides as well as certain arginine derivatives are clearly effective (1). The threshold concentration for endothelium-dependent relaxation by most of the known agonists is in the nanomolar to low micromolar range. Moreover, the effect of both L- and D-arginine is potentiated but not mediated by the endothelium. Recently an endothelium-dependent relaxation (9% maximum relaxation; n = 3) was reported for L-arginine but not D-arginine (12). Our results show that irrespective of the stereochemistry or the presence of endothelium all basic amino acids and not only arginine will elicit relaxation, although at very high doses. Similar results using both D- and L-arginine were also reported by Vane *et al.* (13) which attributed their vasodilating effect to a change in pH. Taken together, these findings raise doubts whether the endogenous precursor of EDRF is indeed L-arginine or another simple basic amino acid. The release of NO from macrophages using L-arginine required a considerable lag period (> 12 h) to generate any detectable amount of NO/NO_2^- (6) which might be considered as indicating that the endogenous precursor is an arginine containing moeity as we have proposed previously (3). Such arginine containing compounds could be stored in the endothelium and released upon stimulation and calcium mobilization.

In summary, our results suggest that polycations like poly-L-arginine elicit endothelium-dependent relaxation at submicromolar concentrations by interaction with anionic sites present in the luminal surface of the endothelium and subsequent EDRF release by a yet unknown mechanism. Irrespective of their stereochemistry, basic amino acids such as arginine are also capable to induce relaxation, but at probably non-physiological concentrations and not exclusively mediated by the endothelium. This effect may be due to a change in the pH of the medium. In view of the aforementioned findings, recent reports stating that L-arginine is the physiological precursor of EDRF appear to be questionable.

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