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Effect of L-arginine and an Arginine-Containing Pentapeptide on Canine Femoral Arterial Blood Flow

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ABSTRACT

The amino acid L-arginine is a precursor of endothelium derived relaxing factor (EDRF). The pentapeptide 6A (Ala-Arg-Pro-Ala-Lys) released by plasmin degradadation of fibrinogen also contains arginine and relaxes vascular smooth muscle by releasing EDRF (nitric oxide). To determine and compare the effects of L-arginine, peptide 6A and a combination of L-arginine and peptide 6A on femoral artery blood flow and vascular resistance, anesthetized mongrel dog were administered saline, L-arginine, D-arginine, peptide 6A and L-arginine + peptide 6A in a random order.

L-arginine and peptide 6A both induced an immediate dose-dependent short-lasting increase in femoral blood flow and a decrease in vascular resistance. Peptide 6A exerted a much greater ($P<0.01$) vasodilatory effect than did L-arginine at the same molar concentration suggesting that properties besides the arginine content are important in the effect of the pentapeptide. D-arginine had much less effect than L-arginine, indicating that the effect of L-arginine may be related to its utilization for synthesis of EDRF. When the peptide 6A was given soon after L-arginine, its effect on blood flow was not greater than that of L-arginine alone suggesting that L-arginine in a large amount makes guanylate cyclase less available for the more active peptide.

INTRODUCTION

Vascular endothelial cells synthesize nitric oxide from the terminal guanido nitrogen atom(s) of L-arginine and this accounts for the

biological activity attributed to endothelium-derived relaxing factor (EDRF) (7). We have observed that a pentapeptide, Ala-Arg-Pro-Ala-Lys, released by plasmin degradation of fibrin(ogen) increases coronary arterial blood (5). As indomethacin did not abolish this increase, the pentapeptide may possibly cause release of EDRF, as the release of this substance is not inhibited by indomethacin (3). The purpose of the present study was to determine whether intraarterial administration of L-arginine and the arginine containing pentapeptide altered the femoral arterial blood flow and vascular resistance in the dog and, if so, to compare the effect of each agent given alone with that of the two agents combined. It was thought that such experiments might elucidate the mechanism underlying the influence of peptide 6A.

MATERIAL AND METHODS

Animal preparation

Mongrel dogs weighing 20-25 kg were anesthetized with pentobarbital sodium (30 mg/kg), intubated, and placed on positive pressure ventilation using a Harvard respirator. An ultrasonic Doppler (Valpey-Fisher) or electro-magnetic (Biotronex, BI 613) flow probe was placed on the femoral artery for measurement of femoral arterial blood flow. The artery was kept moist at all times with physiological saline. The femoral arterial pressure was measured by inserting a 3F catheter tip pressure transducer (Millar) into the femoral artery via a side branch and advancing it to the site of flow measurement. Recordings were made on a Gould multichannel recorder. A small-bore catheter was inserted distal to the flow probe through a side branch of the femoral artery and advanced to the main stem of the artery. Its position was checked at the end of the experiment by opening the femoral artery. This catheter was used for infusion of saline, peptide 6A, L-arginine (R-GeneR 10, Kabi Vitrum Inc, Alameda, CA, 10% Arginine HCl), D-arginine (D-arginine Hydrochloride, Sigma Chemical Company, St. Louis, MO, which may contain a small amount of L-arginine according to the manufacturer) or peptide 6A + L-arginine in random order.

Peptide 6A

The pentapeptide, Ala-Arg-Pro-Ala-Lys (Mw=547), named 6A since its

isolation (1) and corresponding to residues 43-47 of the human fibrinogen Bß-chain, was synthesized by the Merrifield solid phase technique . It was isolated in a homogenous state by chromatography on BioGel P-6 and column zone electrophoresis.
Peptide 6A (10, 20 or 50 μmoles), L-arginine (5.5, 55, 555 or 1,100 μmoles) or saline was injected into the femoral artery over 10 seconds. In other experiments, peptide 6A was administered immediately after administration of L-arginine. In a separate experiment L-arginine and D-arginine (5.5, 55 or 550 μmoles) were each infused in random order in three dogs. The volume of the injectate was kept at 0.4 ml.

Statistical analysis

Data are based on 13 experiments in eight dogs and expressed as mean ±SD. Student's t-test and Wilcoxon's nonparametric test were used for comparison of groups.

RESULTS

The basal femoral arterial blood flow varied between 50 and 79 ml/min (mean 51 ± 13) and the femoral vascular resistance between 1.0 and 2.9 mm Hg/ml/min (mean 2.2 ± 0.6). Injection of saline into the femoral artery caused no significant change in femoral artery blood flow or pressure or vascular resistance. Administration of peptide 6A or L-arginine as a bolus into the femoral artery was associated with a prompt increase in the blood flow (Table 1). The blood flow increased with each dose and the increase was dependent on the amount of drug administered. The effects of L-arginine and peptide 6A were qualitatively similar. This was not unexpected since this pentapeptide contains an arginine residue and it is possible that this arginine in peptide 6A is converted to nitric oxide. However, peptide 6A had a much greater ($P<0.01$) effect than L-arginine; 20 μmoles of 6A resulted in almost the same increase in flow as 1,100 μmoles of L-arginine. The pentapeptide seemed to be about 30-40 times more potent on a molar basis. There was no significant change in mean arterial blood pressure during the bolus injections. When 50 μmoles of peptide 6A were administered immediately after 1,100 μmoles of L-arginine there was no further increase in femoral arterial blood flow over and above that caused by

L-arginine alone (Table I). D-arginine had no effect on the flow in one dog and had less effect than L-arginine in the other two dogs.

Table 1.

Effects of Peptide 6A (μmoles), L-arginine (μmoles), and their Combination on Femoral Arterial Blood flow and Vascular Resistance

Mean values ± S.D.
****=p<0.05 **=p<0.01 ***=p<0.001***
compared with saline

		ΔBlood flow (%)	ΔVascular resistance (%)
Peptide 6A	10	42±17*	-32±14
	20	80±36*	-45±16
	50	107±32***	-52±18***
L-arginine	5.5	5±6	-4±2
	55	14±9	-11±7
	555	75±27*	-44±12***
	1,100	81±25*	-49±6***
L-arginine+ Peptide 6A	1,100+20	63±30*	-40±11**
	1,100+50	73±56	-42±18*

DISCUSSION

The finding that peptide 6A had a more potent effect than L-arginine suggests that components other than this amino acid contribute to the effect of the pentapeptide. On the other hand it cannot be excluded that arginine has a special role in peptide 6A. It has been shown that peptides that contain L-arginine at the NH2- or COOH-terminal activate guanylate cyclase, the enzyme responsible for the synthesis of cyclic GMP (2). The alanine at the NH2-terminal in peptide 6A might easily be split off by aminopeptidase in the circulation leaving arginine free at the NH2-terminal. Another vasoactive peptide released by plasmin degradation of fibrin(ogen), Bß 30-43, contains two arginine residues one of which at the NH2-terminal. This peptide (Bß 30-43) indeed has

a more potent vasoactive effect than does peptide 6A (6).

As both peptide 6A and L-arginine increased femoral blood flow, an additive or synergistic effect with their combination might be expected. However, addition with peptide 6A did not result in an increased effect compared to L-arginine alone. The reason for this is not known. It is possible that administration of L-arginine in a large amount makes guanylate cyclase less available for the more active peptide. Infusion of L-arginine may also enhance the basal release of EDRF (4) and thereby reduce the sensitivity to peptide 6A. Importantly, L-arginine caused a larger increase in femoral blood flow than D-arginine. This suggests that the increase in blood flow in response to L-arginine may be related to its utilization for synthesis of nitric oxide. This is in line with a recent report on an increase in the vasodilatory potential of atherosclerotic vessels pre-exposed to L-arginine (4). As D-arginine was not without effect in the present investigation, other mechanisms underlying the increase in blood flow in response to arginine-containing compounds are possible. However, it can not be excluded that the effect of D-arginine was due to contamination of the preparation by a small amount of L-arginine.

In summary, this study shows that peptide 6A and L-arginine have potent vasodilator effects. The greater potency of the effect of peptide 6A (vs L-arginine on a molar basis) suggests that components other than arginine in peptide 6A also contribute to the action of this peptide.

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