Br J clin Pharmac 1994; **38**: 307–310

Effect of N^G-monomethyl-L-arginine on kinin-induced vasodilation in the human forearm

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1 We compared effects of N^G-monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor, on vasodilator responses to intra-arterial infusion of bradykinin and substance P in the human forearm.

2 Bradykinin (100 pmol min^{-1}) increased forearm blood flow when infused into the brachial artery of eight healthy male volunteers, from 2.8 ± 0.2 (mean ± s.e. mean) to 9.3 ± 1.0 ml min^{-1} per 100 ml forearm volume.

3 Co-infusion of L-NMMA (2 μmol min^{-1} and 4 μmol min^{-1}) with bradykinin (100 pmol min^{-1}) for 6 min produced respectively a 9 ± 14% and 42 ± 14% inhibition (compared with L-NMMA vehicle) in the response to bradykinin.

4 Substance P (1 pmol min^{-1}) when infused into the brachial artery of a further eight male subjects increased forearm blood flow from 3.4 ± 0.2 to 6.3 ± 0.7 ml min^{-1} 100 ml^{-1}.

5 Co-infusion of L-NMMA (2 μmol min^{-1} and 4 μmol min^{-1}) with substance P (1 pmol min^{-1}) for 6 min produced respectively a 27 ± 8% and 70 ± 13% inhibition (compared with L-NMMA vehicle) in the response to substance P.

6 These results demonstrate that vasodilator responses to both bradykinin and substance P are mediated in part via the L-arginine/NO pathway. Bradykinin and substance P may be useful agonists with which to study endothelial function in this vascular bed.

Keywords endothelium nitric oxide N^G-monomethyl-L-arginine bradykinin substance P human forearm vasculature

Introduction

The activity of several vasodilators depends on the integrity of the vascular endothelium [1]. These include acetylcholine [2], bradykinin [3] and substance P [4]. An important component of such endothelium-dependent responses consists of Ca^{2+}-dependent stimulation of a constitutive NO synthase that catalyses conversion of L-arginine to L-citrulline and NO [5]. NO diffuses from endothelial cells to underlying smooth muscle where it activates guanylyl cyclase and causes relaxation [6]. There has been much interest in the possibility that pathological conditions that are risk factors for cardiovascular disease, including diabetes, hypertension and hypercholesterolaemia, operate in part through impairment of the L-arginine/NO pathway. As well as relaxing vascular smooth muscle NO also inhibits platelet function [7] and vascular smooth muscle proliferation [8], suggesting that impairment of its biosynthesis could predispose to arterial thrombosis and atherogenesis. Endothelium dependent agonists have therefore been widely used as pharmacological tools to study a variety of physiological and pathophysiological conditions. Results from such studies have sometimes been conflicting and difficult to interpret. An explanation for this may be due to the fact that the various agonists used vary not only in the amount of their action that is mediated via the L-arginine/NO pathway but also in their receptor coupling to this pathway.

N^G-monomethyl-L-arginine (L-NMMA) an inhibitor of NO synthase [6], partially inhibits responses to acetylcholine and carbachol (a stable analogue of

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acetylcholine) in human forearm resistance vessels [9, 10] but is ineffective against methacholine, another relatively stable analogue of acetylcholine [11].

In vitro data suggest that kinins may also be useful probes for the investigation of endothelial function in the human forearm vascular bed. Therefore in the present study we have investigated the effect of L-NMMA on vasodilator responses to kinins, comparing effects of this inhibitor on vasodilator responses to bradykinin and substance P.

Methods

Studies were performed on healthy male volunteers aged 22–28 years. All subjects gave written informed consent. The study was approved by the local ethics committee.

Eight subjects were each studied using bradykinin on two separate occasions, and a further eight similarly received substance P. Drugs were obtained from Clinalfa, AG, Switzerland and prepared as previously described [10]. Experiments were performed in a temperature controlled laboratory (22–24° C). Forearm blood flow (ml min^{-1} per 100 ml forearm volume) was measured in both arms using venous occlusion plethysmography [12] with electrically calibrated temperature compensated mercury in silastic strain gauges [13]. During measurement periods the hands were excluded from the circulation by means of wrist cuffs inflated to a pressure of 180 mm Hg. Upper arm cuffs were inflated intermittently to a pressure of 40 mm Hg to prevent venous return. A 27 gauge unmounted steel needle (Coopers Needle Works, Birmingham UK) was inserted into the left brachial artery for infusion of drugs or saline. All infusions were at a constant rate of 1 ml min^{-1}. On each occasion subjects received an intra-arterial infusion of saline for 12 min followed by an 18 min infusion of kinin (bradykinin 100 pmol min^{-1}, or substance P, 1 pmol min^{-1}). On one occasion L-NMMA (2 and 4 μmol min^{-1}) was co-infused with kinin during the final 12 min, each dose for 6 min. On the other occasion saline was co-infused instead of L-NMMA. Forearm blood flow was measured during infusion of saline alone at the end of the baseline period in every study, during infusion with kinin alone (from 3–6 min), and during the final 3 min of the period of co-infusion with either saline or L-NMMA (2 and 4 μmol min^{-1}). Flows were recorded for 10 s in every 15 s and the mean of the final five measurements of each recording period used for analysis.

Statistical analysis

Results are presented as means (± s.e. mean). Changes in vasodilator responses due to either L-NMMA or saline were expressed as a percentage of the blood flow response during agonist administration immediately before co-infusion. Data were analysed by analysis of variance for repeated measures. Differences were considered significant when $P < 0.05$.

Results

Blood flow in the control (non-infused) arm did not alter significantly throughout the experimental period on any study day, confirming that at the doses infused bradykinin, substance P and L-NMMA did not cause systemic effects. Bradykinin (100 pmol min^{-1}) alone increased blood flow from 3.2 ± 0.3 to 9.4 ± 1.0 ml min^{-1} 100 ml^{-1} on the saline day and from 2.8 ± 0.2 to 9.3 ± 1.0 ml min^{-1} 100 ml^{-1} on the L-NMMA day (Table 1). Table 1 also shows the percentage change in response to kinin produced by co-infusion of either saline or L-NMMA relative to the baseline period of kinin infusion. Co-infusion of L-NMMA inhibited the response to bradykinin relative to saline control by 9 ± 14% (2 μmol min^{-1}, $P = 0.21$) and 42 ± 14% (4 μmol min^{-1}, $P < 0.001$) (Figure 1).

Substance P alone increased forearm blood flow from 3.0 ± 0.2 to 5.6 ± 0.5 ml min^{-1} 100 ml^{-1} on the saline day and from 3.4 ± 0.2 to 6.3 ± 0.7 ml min^{-1} 100 ml^{-1} on the L-NMMA day (Table 1). Responses to substance P relative to saline were inhibited by co-infusion of L-NMMA by 27 ± 8% at a dose of 2 μmol min^{-1} ($P < 0.01$) and by 70 ± 13% at a dose of 4 μmol min^{-1} ($P < 0.001$) (Figure 1). Responses to both bradykinin and substance P were unaffected by co-infusion of saline (Figure 1).

Table 1 Effect of N^G-monomethyl-L-arginine (L-NMMA) or saline on vasodilator responses to bradykinin (BK) and substance P (SP)

	Forearm blood flow (ml min^{-1} 100 ml^{-1})							
Study day	*Baseline*	*Kinin*	*Kinin with saline/L-NMMA(2)*†	*Kinin with saline/L-NMMA(4)*	*% change with saline/L-NMMA(2)*		*% change with saline/L-NMMA(4)*	
BK/saline	3.2 ± 0.3	9.4 ± 1.0	9.1 ± 0.9	9.8 ± 1.0	0 ± 8	NS	11 ± 6	*
BK/L-NMMA	2.8 ± 0.2	9.3 ± 1.0	8.5 ± 0.99	7.1 ± 0.8	−9 ± 10		−31 ± 9	
SP/Saline	3.0 ± 0.2	5.6 ± 0.5	5.6 ± 0.5	5.7 ± 0.5	0 ± 3	*	3 ± 4	**
SP/L-NMMA	3.4 ± 0.2	6.3 ± 0.7	5.7 ± 0.7	4.8 ± 0.6	−27 ± 10		−67 ± 16	

*P < 0.01, **P < 0.001, NS = not significant.
†L-NMMA (dose in μmol min^{-1}).

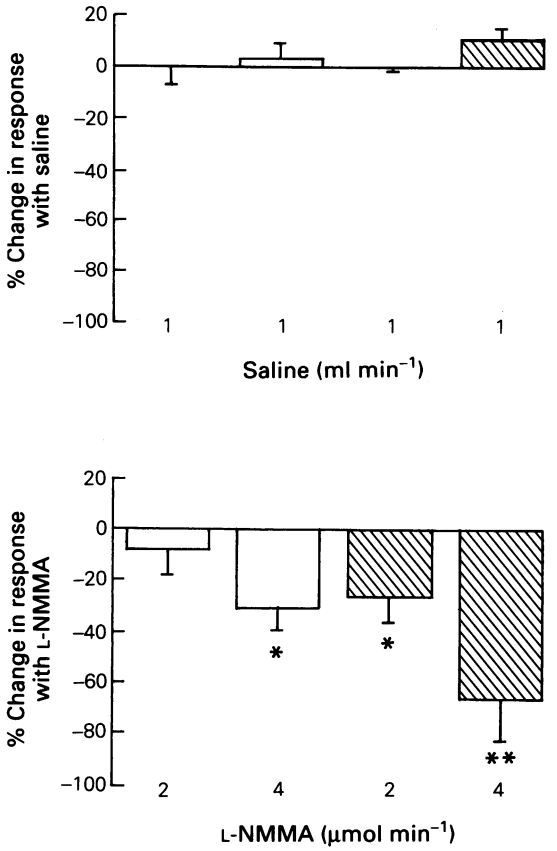

Figure 1 Percentage change in response to bradykinin (100 pmol min^{-1}, open bars) and substance P (1 pmol min^{-1}, hatched bars) after 6 min co-infusions of saline (upper panel) or N^G-monomethyl-L-arginine (L-NMMA) 2 and 4 μmol min^{-1} (lower panel). $*P < 0.01$, $**P < 0.001$ with respect to saline.

Discussion

The main finding of this study is that L-NMMA inhibits vasodilation caused by kinins in a dose dependent manner. The finding that the vasodilator response to bradykinin in the human forearm vascular bed can be inhibited by co-infusion of L-NMMA is in agreement with preliminary observations of Vallance and colleagues [14], and with observations in human dorsal hand veins *in vivo* [15]. Vasodilator responses to bradykinin in this vascular bed are not inhibited by the cyclooxygenase inhibitor aspirin [16], suggesting bradykinin is not acting via increased endothelial prostacyclin release. The finding that bradykinin exerts some of its vasodilator action via the L-arginine/NO pathway may be of clinical importance as inhibition of angiotensin converting enzyme (ACE) inhibits the breakdown of bradykinin [17] increasing its potency [16]. Enhanced NO release could therefore contribute to the beneficial effects of ACE inhibition [18].

Substance P is an 11-amino acid peptide which is found in both central and peripheral nerves. It produces potent vasodilation in both the human coronary [19] and forearm circulations [20, 21] and reduces total peripheral resistance during intravenous infusion [22]. Substance P is the endogenous ligand at tachykinin (TK)-1 receptors [23] and causes vasorelaxation via an endothelium-dependent mechanism [24] which can be inhibited by L-NMMA but not D-NMMA [25]. The effect of a specific inhibitor of nitric oxide synthesis on the actions of substance P in a human vascular bed has not been studied previously. Our results show that L-NMMA produces dose related inhibition of the vasodilator response to substance P in the forearm vasculature.

Acetylcholine has been widely used as an endothelium-dependent vasodilator to study the integrity of the L-arginine/NO pathway in the human forearm [26, 27]. Responses to acetylcholine in this vascular bed are partially inhibited by L-NMMA (4 μmol min^{-1}) by between 40–60% [9, 11]. In the present study kinin induced vasodilation was inhibited to a similar degree by L-NMMA. The sites in the L-arginine/NO pathway at which responses to NO dependent vasodilators are impaired in vascular disease remain unclear. Specific defects may exist at either the level of the receptor, G-proteins or more distal transduction mechanisms [28]. Kinins acting through different transduction mechanisms to acetylcholine [23] may therefore prove useful tools in defining the site of specific defects within the L-arginine/NO pathway. For example in the coronary circulation responses to acetylcholine but not to substance P are impaired in atherosclerosis [19].

In conclusion this study has shown that kinin-induced vasodilation is mediated in large part through the L-arginine/NO pathway. Kinins may provide additional insights into endothelial dysfunction in cardiovascular disease states.

This work was supported in part by the British Heart Foundation.

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(Received 25 January 1994, accepted 17 June 1994)