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Persistence of effects of nitric oxide synthase inhibitors: Comparisons on blood flow and plasma exudation in guinea pig skin

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Abstract

Plasma protein extravasation has been measured in guinea pig skin using ^{125}I-albumin and blood flow using ^{133}Xenon (^{133}Xe) clearance. The nitric oxide (NO) synthase inhibitors N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (l-NMMA) and N^G-nitro-L-arginine (L-NOArg) and the α-adrenoceptor agonist, phenylephrine, inhibited bradykinin induced plasma protein extravasation when co-injected with the peptide. The inhibitory effects of L-NAME and L-NOArg lasted for up to 8 and 4 h, respectively, whereas phenylephrine and L-NMMA had no persistent inhibitory effects. When co-injected with ^{133}Xe, L-NAME, L-NMMA, L-NOArg and phenylephrine, but not D-NAME, produced significant reductions in skin blood flow. When injected prior to ^{133}Xe, L-NAME and L-NOArg, but not phenylephrine or L-NMMA, significantly reduced flow. The effect of L-NAME on flow was not significant at 8 h. Thus, although the inhibitory effects of the NO synthase inhibitors on mediator induced plasma protein extravasation show correlations with their effects on blood flow, the persistent effect of L-NAME on exudation appears to extend beyond its effect on flow. © 1997 Elsevier Science B.V.

Keywords: NO synthase inhibitors; Plasma leakage; Blood flow; Bradykinin; Histamine

1. Introduction

Oedematous responses in cutaneous inflammation have been suggested to be dependent on interactions between mediators that increase vascular permeability and those that can produce alterations in blood flow (Williams and Morley, 1973; Williams and Peck, 1977). Vascular endothelium can generate both vasodilator agents, such as prostaglandins E_2 and I_2 and nitric oxide (NO) and constrictor agents, such as endothelin (Thiemermann, 1991), which may act as local modulators of oedema formation.

A pro-inflammatory role for NO can be inferred from the ability of several NO synthase inhibitors to significantly reduce plasma exudation and oedema in a variety of inflammatory responses (Antunes et al., 1990; Hughes et al., 1990; Ialenti et al., 1992; Khalil and Helme, 1992; Mulligan et al., 1992; Paul et al., 1992; Kurose et al., 1993; Teixeira et al., 1993; Paul et al., 1994). In some of these studies, the NO synthase inhibitors were also shown to have the capacity to produce significant reductions in blood flow at doses which reduced plasma leakage (Hughes et al., 1990; Khalil and Helme, 1992; Teixeira et al., 1993). The evidence, therefore, suggests that endogenous NO causes vasodilatation which augments oedema formation and that NO synthase inhibitors, by blocking production of NO, reduce local blood flow and hence exert an anti-oedema effect. However, the NO synthase inhibitors N^G-nitro-L-arginine methyl ester (L-NAME) and 7-nitro indazole (Moore et al., 1993) have been shown to inhibit neurogenic oedema in rats in the absence of significant effects on blood flow (Kajekar et al., 1995). Moreover, prolonged effects of NO synthase inhibitors have been reported in the mouse, where L-NAME exerted an antinociceptive effect which was still present 24 h after injection (Moore et al., 1991).

We have, therefore, studied the time course of the effects of NO synthase inhibitors on cutaneous plasma protein extravasation and blood flow in guinea pig skin in

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order to further address the relationship between these two phenomena.

2. Materials and methods

2.1. Experimental animals

Conscious male, Dunkin Hartley guinea-pigs (400–500 g body weight) were used throughout. The hair on the skin of the back and flanks was closely shaved with clippers at least 2 h before each experiment.

2.2. Intravenous injections

Guinea pigs were injected via a hind paw dorsal vein with 0.5 ml Evans blue dye (2.5% w/v) in sterile phosphate buffered saline (PBS) containing approximately 0.07 MBq ^{125}I labelled human serum albumin.

2.3. Intradermal injections

Test solutions were prepared in sterile PBS immediately prior to use. Intradermal injections (0.1 ml/site) were made according to a balanced Latin square design to allow for inter-site variation. Each animal received up to 6 treatments in duplicate. PBS and drug alone controls were included in each experiment.

2.4. Preparation of skin samples

At the end of experiments (40 min after the i.d. injections), the animals were killed by an overdose of pentobarbitone sodium administered i.p., a blood sample was taken by cardiac puncture for preparation of plasma and the skin was removed. Each site of the skin was punched out using a 17 mm metal wad punch and, together with duplicate 100 μl plasma samples, counted for ^{125}I content in an automated gamma spectrometer.

2.5. Calculation of results

Plasma protein extravasation was expressed as μl of plasma, calculated as ^{125}I counts in skin sample/ ^{125}I counts in 1 μl plasma. These values were corrected by subtracting the control response for PBS or test drug alone, as appropriate. Results are presented as mean ± S.E.M. values for the stated number of animals. Data were analysed by analysis of variance followed by Tukey's test to allow for multiple comparisons

2.6. Blood flow measurements

Changes in cutaneous blood flow were measured using a multiple site ^{133}Xenon (^{133}Xe) clearance method (Williams, 1979). Sites on the shaved flank of each animal were injected with 0.1 ml volumes containing approximately 0.08 MBq ^{133}Xe. Injections of ^{133}Xe, diluted in PBS, were made at various times after the i.d. injection of the drug under test (pretreatment) or of ^{133}Xe diluted in a solution containing the test drug (co-injection). After 15 min animals were killed by i.v. injection of an overdose of pentobarbitone sodium and then skinned. Discs of skin containing the injection sites were removed with a wad punch as for plasma protein extravasation measurements. The skin samples and 0.1 ml samples of the injection solutions were placed under 2 ml liquid paraffin in capped vials and counted for ^{133}Xe in an automatic gamma spectrometer. Results were calculated as % change in ^{133}Xe clearance in test sites compared with control (saline treated) sites (Williams, 1979).

Results were calculated using the equation:

$$\left(\left[\ln^{133}\mathrm{Xe}_S - \ln^{133}\mathrm{Xe}_A\right] \times 100\right) / \left[\ln^{133}\mathrm{Xe}_1 - \ln^{133}\mathrm{Xe}_S\right]$$

= % change in ^{133}Xe clearance relative to control, saline-injected sites,

where $^{133}\mathrm{Xe}_S$ = count/min in saline-injected skin, $^{133}\mathrm{Xe}_A$ = count/min in test-agent-injected skin and $^{133}\mathrm{Xe}_1$ = count/min in 0.1 ml volume of injection fluid.

2.7. Materials

Histamine diphosphate, bradykinin acetate, Evans blue dye, sterile phosphate buffered saline, L-NAME, N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NOArg), L-arginine HCl, phenylephrine HCl and pentobarbitone sodium were obtained from Sigma (Poole, UK) and D-NAME from Bachem (Buchs, Switzerland). ^{125}I-human serum albumin (1.85 MBq/ml; 20 mg albumin/ml) was supplied by Amersham International (Amersham, UK) and ^{133}Xenon (74 MBq/ml) by Medgenix (High Wycombe, UK).

3. Results

3.1. Inhibitory effects of L-NAME on skin plasma exudation

L-NAME (0.1 μmol/site) was injected, in separate experiments, at times ranging from 0.5–16 h prior to the injection of bradykinin (0.75 nmol/site) to assess the duration of its inhibitory effect on plasma exudation. In each experiment, the effect of co-injection of L-NAME was tested and control sites were pre-injected with PBS at the appropriate time. Inhibition of bradykinin induced plasma exudation by co-injection of L-NAME was 57.6 ± 2.9% in 6 experiments. Inhibition in sites injected with L-NAME 0.5 h prior to bradykinin was significantly greater ($P < 0.05$; $n = 6$) than that produced in the co-injected sites (Fig. 1). The significant inhibitory effects of L-NAME 1 or 2 h after injection (both $P < 0.01$; $n = 6$) were greater

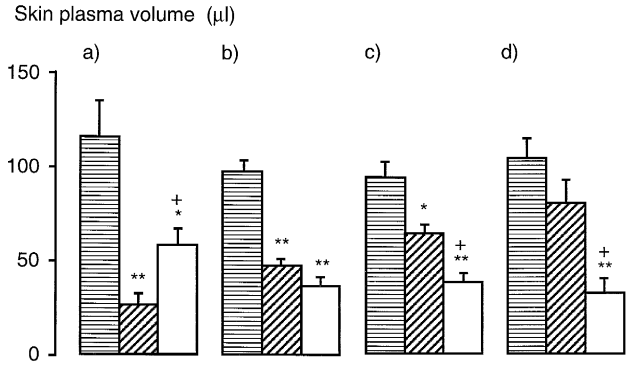

Fig. 1. Persistence of the effect of L-NAME (0.1 μmol/site) on the plasma exudation response to bradykinin (0.75 nmol/site). Responses to the mediator alone (horizontal and diagonal hatched columns) or mixed with L-NAME (open columns) were measured (a) 0.5, (b) 4, (c) 8 and (d) 16 h after injection of saline (horizontal hatched and open columns) or L-NAME (diagonal hatched columns). Extravasation responses are expressed as μl plasma (corrected values) and each column represents the mean value with vertical bars indicating 1 S.E.M. $n = 6$ throughout. * $P < 0.05$, ** $P < 0.01$ versus corresponding mediator alone values. $^{+}$ $P < 0.05$ versus corresponding preinjection values.

than those produced by co-injection, although the differences between pre- and co-injection were not statistically significant (data not shown). At 8 h following injection, the inhibitory effect of L-NAME was still significant ($P < 0.05$; $n = 6$) but was now significantly ($P < 0.05$) less than co-injection and inhibition was no longer significant at 16 h (Fig. 1). A similar duration for the inhibitory effect of L-NAME (0.1 μmol/site) was also found using histamine (5.4 nmol/site) as the permeability-inducing mediator (Table 1).

When the dose of L-NAME was reduced to 0.02 μmol/site, co-injection produced 41.3 ± 5.4% inhibition of bradykinin (0.75 nmol/site) induced extravasation in 3 experiments (Table 2). Sites injected with the low dose of L-NAME 0.5 or 2 h prior to bradykinin showed significant ($P < 0.01$; $n = 6$) inhibition of extravasation whereas the effect was no longer significant at 4 h (Table 2). This

Table 1
Persistence of effect of L-NAME on histamine-induced skin plasma protein exudation

	Time of pre-injection (h)			
	0.5	4	8	16
HA	83.6 ± 9.9	76.6 ± 4.4	72.5 ± 6.5	97.3 ± 4.9
PRE	15.5 ± 2.0 [b]	35.8 ± 4.7 [b]	50.4 ± 4.5 [a]	96.8 ± 11.3
CO	36.2 ± 6.6 [a]	31.7 ± 3.6 [a]	31.7 ± 2.2 [a,c]	50.2 ± 5.7 [a,d]

Comparison of pre-injection (PRE) with co-injection (CO) of L-NAME (0.1 μmol/site) on exudation responses to histamine (HA, 5.4 nmol/site). Sites were injected with saline (HA and CO) or with L-NAME (PRE) at the specified times prior to injection of mediator (HA and PRE) or mediator + L-NAME (CO). Values are mean ± S.E.M. skin plasma volume (μl) corrected by subtracting appropriate control (blank) values. $n = 5$–6 throughout.
[a] $P < 0.05$, [b] $P < 0.01$ versus corresponding mediator control; [c] $P < 0.05$, [d] $P < 0.01$ versus corresponding pre-injection values.

Table 2
Persistence of effect of low dose L-NAME on bradykinin-induced skin plasma protein exudation

	Time of pre-injection (h)		
	0.5	2	4
BK	121.0 ± 11.8	101.0 ± 9.0	75.7 ± 6.7
PRE	53.5 ± 7.7 [b]	61.1 ± 9.2 [b]	58.8 ± 5.0
CO	84.2 ± 6.8 [a]	53.5 ± 4.5 [b]	40.6 ± 5.5 [b]

Comparison of pre-injection (PRE) with co-injection (CO) of L-NAME (0.02 μmol/site) on exudation responses to bradykinin (BK, 0.75 nmol/site). Sites were injected with saline (BK and CO) or with L-NAME (PRE) at the specified times prior to injection of mediator (BK and PRE) or mediator + L-NAME (CO). Values are mean ± S.E.M. skin plasma volume (μl) corrected by subtracting appropriate control (blank) values. $n = 6$ throughout.
[a] $P < 0.05$, [b] $P < 0.01$ versus corresponding mediator alone values.

lower dose of L-NAME did not significantly reduce the response to histamine (5.4 nmol/site; 72.5 ± 6.5 μl) when co-injected (50.4 ± 4.5 μl) but produced a significant ($P < 0.05$; $n = 6$) inhibition when injected 0.5 h before histamine (31.7 ± 2.2 μl).

3.2. Effects of D-NAME and phenylephrine on skin plasma exudation

D-NAME (0.1 μmol/site) had no significant effect on bradykinin (0.75 nmol/site) induced extravasation when co-injected with, or injected 0.5 h prior to, the permeability mediator (Fig. 2a). The small effect seen with 0.5 h pre-injection was no longer evident by 2 h (saline pre-injected controls: 103.3 ± 9.1 μl; D-NAME pre-injection: 90.1 ± 10.4 μl; $n = 6$).

Phenylephrine (5 nmol/site) reduced the exudation response to bradykinin (0.75 nmol/site) significantly ($P <$

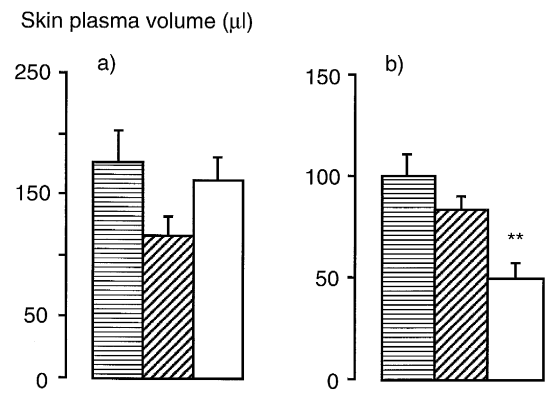

Fig. 2. Effects of (a) D-NAME (0.1 μmol/site; $n = 6$) or (b) phenylephrine (5 nmol/site; $n = 4$) on plasma exudation responses to bradykinin (0.75 nmol/site). Responses to the mediator alone (horizontal hatched columns) or D-NAME or phenylephrine injected 0.5 h prior to (diagonal hatched columns) or co-injected with (open columns) bradykinin are expressed as μl plasma (corrected values). Each column represents the mean value with vertical bars indicating 1 S.E.M. ** $P < 0.01$ versus corresponding mediator alone values.

Table 3
Persistence of effects of L-NMMA and L-NOArg on skin plasma exudation

	Time of pre-injection (h)		
	0.5	2	4
(a)			
BK	83.7 ± 9.0	112.1 ± 14.1	94.5 ± 12.0
PRE	65.0 ± 15.4	100.8 ± 6.2	98.2 ± 9.6
CO	47.0 ± 4.1 [a]	74.2 ± 8.5 [a,c]	51.1 ± 6.1 [b,d]
(b)			
BK	70.3 ± 1.9	126.2 ± 12.6	83.5 ± 7.6
PRE	29.4 ± 4.7 [a]	73.7 ± 8.8 [b]	53.2 ± 9.2 [a]
CO	44.2 ± 3.4 [a,c]	57.5 ± 7.4 [b]	24.2 ± 1.9 [b,c]

Comparison of pre-injection (PRE) with co-injection (CO) of 0.1 μmol/site of (a) L-NMMA or (b) L-NOArg on the exudation response to bradykinin (BK, 0.75 nmol/site). Sites were injected with saline (BK and CO) or with L-NMMA or L-NOArg (PRE) at the specified times prior to injection of bradykinin (BK and PRE) or bradykinin + L-NMMA or L-NOArg (CO). Values are mean ± S.E.M. skin plasma volume (μl) corrected by subtracting appropriate control (blank) values. $n = 6$ throughout.
[a] $P < 0.05$, [b] $P < 0.01$ versus corresponding mediator alone values.
[c] $P < 0.05$, [d] $P < 0.01$ versus corresponding pre-injection values.

0.01) when co-injected (bradykinin control: 94.2 ± 7.5 μl; bradykinin + phenylephrine: 38.2 ± 7.4 μl; $n = 5$) but not when injected 1 h before the mediator (101.4 ± 17.1 μl). Nor did the same dose of phenylephrine have any significant effect when injected 0.5 h prior to bradykinin (Fig. 2b).

3.3. Effects of other NO synthase inhibitors on skin plasma exudation

The NO synthase inhibitors L-NMMA and L-NOArg (0.1 μmol/site), when co-injected with bradykinin (0.75 nmol/site), produced 43.4 ± 2.5% ($n = 5$ experiments) and 53.3 ± 5.4% ($n = 5$ experiments) inhibition, respectively. The duration of their inhibitory effects was assessed in the same way as for L-NAME. The inhibitory effect of L-NMMA was not significant at 0.5 h (Table 3a) whereas pre-injection of L-NOArg caused significant inhibition of the bradykinin response at times up to 4 h (Table 3b). The inhibitory effect of L-NOArg was significantly ($P < 0.05$) greater when injected 0.5 h before bradykinin than when co-injected (Table 3b).

3.4. Effects of L-arginine on L-NAME inhibition of skin plasma exudation

Injection of L-arginine (10 μmol/site) 2 h before bradykinin (0.75 nmol/site) had no significant effect on the extravasation response (PBS–bradykinin: 94.3 ± 10.4 μl; L-arginine–bradykinin: 99.6 ± 11.5 μl; $n = 6$). When L-arginine was co-injected with L-NAME (0.1 μmol/site) it reduced ($P < 0.05$) the latter's inhibitory effect on bradykinin induced exudation measured 2 h later (PBS–bradykinin: 84.2 ± 3.4 μl; L-NAME–bradykinin: 25.6 ± 3.3 μl; L-NAME + L-arginine–bradykinin: 48.0 ± 4.7 μl; $n = 6$).

The response to bradykinin (PBS–bradykinin: 71.7 ± 5.0 μl; $n = 6$) was significantly ($P < 0.05$) increased by co-injection of L-arginine (PBS–bradykinin + L-arginine: 99.1 ± 9.7 μl) whereas the inhibitory effect produced by injecting L-NAME 2 h before bradykinin (L-NAME–bradykinin 35.4 ± 5.4 μl; $P < 0.01$) was not significantly reversed (L-NAME–bradykinin + L-arginine: 55.6 ± 5.1 μl). When the pre-injection time was extended to 4 h, the response to bradykinin (PBS–bradykinin: 94.0 ± 7.7 μl; $n = 6$) was again significantly ($P < 0.01$) reduced by L-NAME (L-NAME–bradykinin: 38.0 ± 2.4 μl) and co-injection of L-arginine with bradykinin now not only significantly ($P < 0.05$) increased the control response to bradykinin (PBS–bradykinin + L-arginine: 123.4 ± 6.3 μl) but also significantly ($P < 0.01$) reversed the inhibitory effect of L-NAME (L-NAME–bradykinin + L-arginine: 80.7 ± 5.5 μl).

3.5. Effects on skin blood flow

When co-injected with ^{133}Xe, D-NAME (0.1 μmol/site) had no significant effect on blood flow whereas L-NAME (0.1 μmol/site) and phenylephrine (5 nmol/site) produced significant reductions in flow ($P < 0.01$ and $P < 0.001$, respectively; $n = 5$; Fig. 3a). In sites injected 0.5 h prior to injection of ^{133}Xe (in PBS), phenylephrine (5 nmol/site) no longer had a significant effect on blood flow whilst the effects of D- and L-NAME (0.1 μmol/site) were increased, although only the effect of L-NAME was statistically significant ($P < 0.05$; $n = 6$; Fig. 3b).

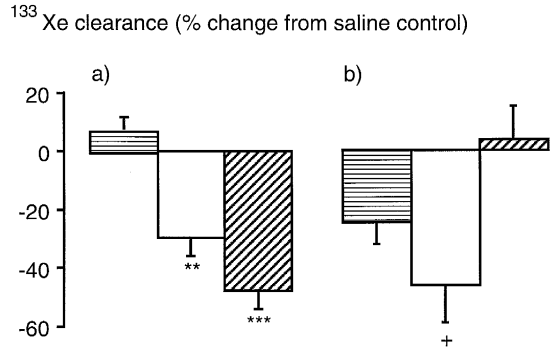

Fig. 3. Effects of (a) co-injection or (b) 0.5 h pre-injection of D-NAME (0.1 μmol/site; horizontal hatched columns), L-NAME (0.1 μmol/site; open columns) and phenyleprine (5 nmol/site; diagonal hatched columns) on skin blood flow measured as ^{133}Xe clearance. Sites were injected with 0.1 μl volumes of saline containing (a) the specified amount of drug and 0.08 MBq of ^{133}Xe or (b) the specified amounts of drug 0.5 h prior to injection of 0.1 ml volumes of saline containing 0.08 MBq ^{133}Xe. Clearance was measured over 15 min following injection of ^{133}Xe and expressed as percentage change compared with saline treated sites. Each column represents the mean value with vertical bars indicating 1 S.E.M. ** $P < 0.01$, *** $P < 0.001$ ($n = 5$) versus corresponding D-NAME values. + $P < 0.05$ ($n = 6$) versus corresponding phenylephrine values.

Table 4
Effect of NO synthase inhibitors on ^{133}Xenon clearance

	Time of pre-injection (h)		
	0	0.5	2
L-NAME	-35 ± 6	-46 ± 2	-36 ± 3
L-NOArg	-30 ± 5	-37 ± 3	-28 ± 3
L-NMMA	-30 ± 5	$-17 + 3$ [a]	-8 ± 4 [a]

Effect of 0.1 μmol/site of L-NAME, L-NOArg and L-NMMA on skin blood flow measured as ^{133}Xe clearance. Sites were injected with 0.1 ml volumes of saline or NO synthase inhibitor at the specified times prior to injection of 0.1 ml saline containing ^{133}Xe. Clearance was measured over a 15 min period. Values are mean ± S.E.M. percentage change in ^{133}Xe clearance relative to PBS controls. $n = 6$ for each time point.
[a] $P < 0.01$ relative to corresponding values for L-NOArg or L-NAME.

In a second series of experiments, the effects of the NO synthase inhibitors L-NMMA and L-NOArg on skin blood flow were compared with those of L-NAME (all compounds at 0.1 μmol/site). As can be seen in Table 4, all of the NO synthase inhibitors produced similar reductions in blood flow when co-injected with ^{133}Xe. When blood flow was measured 0.5 h after injection, L-NMMA no longer had a significant effect whilst the effects of L-NAME and L-NOArg were greater than those produced by co-injection (Table 4). At 2 h, the reductions in flow produced by L-NAME and L-NOArg were similar and were significantly greater than that produced by L-NMMA (Table 4). However, 8 h following injection of L-NAME no measurable reduction in blood flow was seen (PBS: $3.0 \pm 10.0\%$; L-NAME: $8.2 \pm 11.0\%$; $n = 6$). In the same animals, blood flow ($-37 \pm 7\%$) measured immediately after injection of L-NAME was significantly ($P < 0.05$) reduced.

4. Discussion

The observation that co-injection of the NO synthase inhibitor, L-NAME, with bradykinin produces significant inhibition of the plasma protein exudation response in guinea-pig skin (Teixeira et al., 1993; Paul et al., 1994) has been extended to include the inhibitors L-NMMA and L-NOArg which have been shown previously to inhibit vascular permeability induced by substance P (Hughes et al., 1990), carrageenan (Ialenti et al., 1992), immune complexes (Mulligan et al., 1992) and bradykinin (Khalil and Helme, 1992) in rats. In addition, our data show that the inhibitory effects of L-NAME and L-NOArg, but not L-NMMA, are greater when they are injected 0.5 h before bradykinin than when co-injected with the nonapeptide and that the inhibitory effect of L-NAME (0.1 μmol/site) persists for up to 8 h, irrespective of whether the inflammatory mediator is bradykinin or histamine. A prolonged effect of L-NAME on oedema formation has not been reported previously, although the long acting β_2-adrenoceptor agonist, salmeterol, has been shown to inhibit exudation in guinea pig skin when injected locally > 6 h prior to bradykinin (Whelan et al., 1993). Whilst L-NAME exerts a prolonged anti-nociceptive effect in mice, this is not attributed to an anti-inflammatory effect since formalin-induced paw oedema is unaffected by (acute) administration of L-NAME (Moore et al., 1991).

The persistent inhibitory effect of L-NAME on plasma protein extravasation is not shown by the a-adrenoceptor agonist, phenylephrine and hence is not a property of all vasoconstrictors. The involvement of the arginine-NO synthase pathway in the prolonged inhibitory effect of L-NAME is suggested by (a) the lack of effect of D-NAME and (b) the ability of an excess of L-arginine, co-injected with L-NAME, to reduce the inhibitory effect of the latter on bradykinin induced exudation measured 2 h later. This cannot be attributed to a persistent enhancing effect of L-arginine since injection of L-arginine 2 h prior to bradykinin has no effect on the exudation response. The ability of L-arginine to 'reverse' the inhibitory effect of L-NAME on plasma protein exudation measured 4 h later suggests that blockade of NO synthase is waning at this time. Our data are consistent with the possibility that L-NAME dissociates slowly from NO synthase and hence the exudation response is reduced until sufficient NO synthase becomes free or is formed de novo. This possibility receives some support from reports that L-NOArg produces irreversible inhibition of rat cerebellar NO synthase (Dwyer et al., 1991) and dissociates slowly from and inactivates NO synthase purified from porcine brain (Klatt et al., 1994). Analogolously, the long lasting inhibitory effect noted with salmeterol (Whelan et al., 1993) may result from prolonged retention at its site of action (Jack, 1991).

The effects of L-NAME and L-NOArg on both extravasation and blood flow are greater when injected 0.5 h prior to response measurement than when co-injected at the time of response measurement whereas the effect of L-NMMA (and phenylephrine) on skin blood flow has disappeared 0.5 h after injection as has its effect on mediator induced exudation. These observations imply that the onset of blockade of NO synthase by L-NAME (and L-NOArg) is slow and, further, suggest that the early phases of the inhibitory effects of the NO synthase inhibitors on exudation are a consequence of reductions in basal skin blood flow. However, the inhibitory effect of L-NAME on extravasation is still significant 8 h following injection, whereas it produces no measurable reduction in blood flow at this time. Although this discrepancy could be a consequence of measurements being made on basal (rather than induced) flow, other workers have also reported inhibitory effects of L-NAME on neurogenic inflammation in rats in the absence of changes in blood flow (Kajekar et al., 1995). Further evidence of a dissociation between effects on blood flow and on plasma exudation is provided by the observation that L-NAME reduces mustard oil-induced vasodilatation in rat paw skin without affecting plasma protein leakage (Lippe et al., 1993).

In conclusion, our data show clearly that local injection of L-NAME in guinea pig skin produces a prolonged inhibition of mediator induced plasma extravasation. Moreover, this inhibitory effect is still significant at a time (8 h) when L-NAME causes no demonstrable reduction in basal skin blood flow. These data are consistent with the possibility that the persistent inhibitory effect of L-NAME on plasma protein exudation is not a consequence of a reduction in blood flow.

Acknowledgements

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