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Role of nitric oxide in the actions of substance P and other mediators of inflammation in rat skin microvasculature

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Abstract

The role of nitric oxide in inflammatory responses to substance P and other mediators of inflammation was examined in rat skin microvasculature in a blister base raised on the hind footpad. Superfusion of substance P (1 μM) over the blister base caused an increase in plasma extravasation and a vasodilator response which was not maintained. N^G-Nitro-L-arginine (100 μM), an inhibitor of nitric oxide biosynthesis, attenuated vasodilatation and plasma extravasation due to substance P. The inactive isomer N^G-nitro-D-arginine was without effect. Neurokinin A (1 μM), 5-hydroxytryptamine (1 μM), ATP (50 μM) and vasoactive intestinal polypeptide (1 μM) elicited vasodilatation, which for vasoactive intestinal polypeptide was maintained even after washout. 5-Hydroxytryptamine and neurokinin A, but not ATP or vasoactive intestinal polypeptide, significantly increased plasma extravasation. Vasodilatation to neurokinin A, 5-hydroxytryptamine and ATP, and the increase in plasma extravasation due to neurokinin A and 5-hydroxytryptamine were unaffected by N^G-nitro-L-arginine (100 μM), whereas vasodilatation due to vasoactive intestinal polypeptide was significantly attenuated. These findings suggest that in rat skin microvasculature in vivo, nitric oxide is involved in vasodilator responses due to substance P and vasoactive intestinal polypeptide, and plasma extravasation due to substance P, but does not contribute significantly to vasodilatation induced by neurokinin A, 5-hydroxytryptamine or ATP, or to plasma extravasation induced by neurokinin A or 5-hydroxytryptamine.

Keywords: Nitric oxide (NO); Endothelium; Microvasculature; Neurogenic inflammation; Substance P; 5-HT (5-hydroxytryptamine, serotonin); ATP

1. Introduction

Nitric oxide acts as a transduction molecule in many biological systems. Much information on the role of nitric oxide in physiological and pathophysiological processes has been derived from the use of selective inhibitors of its biosynthesis from L-arginine, such as N^G-monomethyl-L-arginine, N^G-nitro-L-arginine and N^G-nitro-L-arginine methyl ester (Rees et al., 1989; Dubbin et al., 1990; Moore et al., 1990). In addition to being synthesized by endothelial cells as a mediator of vasodilatation, nitric oxide is released from many other cell types as well as from peripheral non-adrenergic non-cholinergic nerves in some tissues (Gillespie et al., 1989; Moncada and Higgs, 1990). There is increasing evidence to support a role of nitric oxide as a transmitter in the central nervous system (Garthwaite, 1991) and the discovery that N^G-nitro-L-arginine methyl ester has antinociceptive activity in the mouse has led to the suggestion that nitric oxide may have an important function in pain perception (Moore et al., 1991). Part of the axon reflex vasodilatation in rat skin has been shown to be endothelium-dependent (Low et al., 1989) raising the possibility that nitric oxide may be involved in neurogenic inflammation in the periphery.

Neurogenic inflammation comprises a complex series of mechanisms involving the release of peptide transmitters, including substance P and calcitonin gene-related peptide, from primary afferents. In the rat hind footpad blister model, exogenously applied substance P mimics the inflammatory response, producing marked vasodilatation and plasma extravasation (Khalil

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and Helme, 1990). These responses are effected both through a direct action of substance P on microvascular blood vessels, and indirectly through the action of mediators released by substance P from mast cells. Primary afferents may also be involved (Foreman et al., 1983). Nitric oxide could contribute to these events since nitric oxide released from the endothelium accounts for substance P-induced vasodilatation in large blood vessels (Regoli et al., 1988), and since nitric oxide may be released from mast cells (Salvemini et al., 1990). Furthermore, endogenous nitric oxide modulates oedema formation induced by intradermally injected substance P in the skin of rats (Hughes et al., 1990). In rat skin exogenous calcitonin gene-related peptide elicits a long-lasting vasodilator response that is independent of nitric oxide (Ralevic et al., 1992b). In rabbit skin the release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves is nitric oxide-dependent (Hughes and Brain, 1994).

A number of substances in addition to substance P and calcitonin gene-related peptide are involved in neurogenic inflammation. Neurokinin A (a member of the tachykinin family and hence related to substance P) coexists and is coreleased with substance P and calcitonin gene-related peptide from sensory neurones; vasoactive intestinal polypeptide and ATP have been suggested to have roles as transmitters in sensory nerves; both 5-hydroxytryptamine and ATP are constituents of platelets and mast cells in rats and hence may contribute to local inflammatory mechanisms (Keele and Armstrong, 1964; Couture and Cuello, 1984; Mione et al., 1990). Other local sources of these substances include postganglionic sympathetic and parasympathetic nerves and endothelial cells (Burnstock, 1988).

In large blood vessels in vitro nitric oxide acts as the mediator of endothelium-dependent relaxations to a number of vasoactive substances, including 5-hydroxytryptamine, ATP, acetylcholine and substance P, but its role in the microvasculature is less well-defined. It was the aim of the present study to examine the role of nitric oxide in the inflammatory response to vasoactive mediators at the level of rat skin microvasculature in vivo. Some of these results have been published in preliminary form (Ralevic et al., 1992a).

2. Materials and methods

2.1. Animals

Sprague-Dawley rats of either sex with an average weight of 200–250 g were used. Anaesthesia was induced with pentobarbitone sodium, 60 mg kg^{-1} i.p.). Further doses of 15 mg kg^{-1} anaesthetic were administered intermittently to ensure that rats were kept under a constant state of anaesthesia. This method of anaesthesia was previously shown not to alter basal plasma extravasation or sympathetic tone (Khalil and Helme, 1989). Body temperature of the rats was maintained at 37°C. At the end of the experiment animals were killed by barbiturate overdose.

2.2. Blister induction and experimental protocol

A blister was induced in the right hind footpad of the rat by applying a vacuum pressure of −40 kPa for approximately 30 min, using a metal suction cap heated to 40°C. The surface epithelium was removed and the rat's foot secured in a Perspex chamber with inlet and outlet ports. Ringers solution was perfused over the

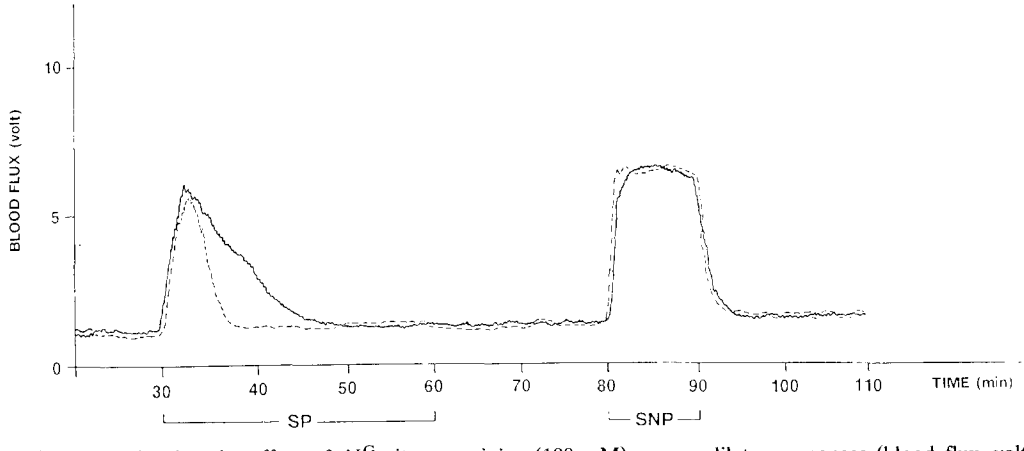

Fig. 1. Representative trace showing the effect of N^G-nitro-L-arginine (100 μM) on vasodilator responses (blood flux, volt) of the rat skin microvasculature to substance P (SP; 1 μM perfused for 30 min) and sodium nitroprusside (SNP; 100 μM perfused for 10 min). Unbroken line indicates control responses. Broken line indicates responses in the presence of N^G-nitro-L-arginine, introduced 14 min prior to and during challenge with substance P or sodium nitroprusside.

surface of the blister at 4 ml h^{-1} by means of a peristaltic pump (Microperpex S, LKB, Sweden) (Khalil and Helme, 1989). Relative blood flow was monitored over time using a laser Doppler flowmeter (Periflux, PF2B, Perimed, Sweden) via a probe placed in a central port immediately above the blister base; blood flux (defined as the number of moving blood cells × mean velocity, and measured in volts) was recorded on a chart recorder (chart speed 0.5 cm min^{-1}). The output signal is linearly related to the flux of red cells; however, the contribution to this signal of small arterioles, capillaries, arteriovenous anastamoses and venules cannot be separated. The influence of variable epidermal thickness on the output signal is not a problem in our preparation since the epithelium is removed.

An initial equilibration period of 30 min was allowed, during which time a stable baseline was achieved. Ringer's solution eluting over the blister base was collected as 10 min fractions and assayed for protein content (Bradford, 1976) as a measure of plasma extravasation. In control experiments substance P (1 μM) was perfused for 30 min over the blister base. In separate experiments neurokinin A (1 μM), 5-hydroxytryptamine (1 μM), ATP (50 μM) or vasoactive intestinal polypeptide (1 μM) were perfused for 20 min over the surface of the blister base. Agonists were used at submaximal concentrations, producing vasodilator responses of approximately 75% of maximum. Times of perfusion were based on the vasodilator characteristics of agonists. Substance P was perfused for 30 min to allow full desensitization to take place and so that the relationship between the declining vasodilatation and plasma extravasation could be examined over time; vasodilator responses to all other agonists were maintained. In experiments using N^G-nitro-L-arginine or N^G-nitro-D-arginine (both at 100 μM) these agents were perfused for 14 min prior to and during perfusion with agonists. At the concentration used N^G-nitro-L-arginine or N^G-nitro-D-arginine did not produce a significant change in the baseline. Sodium nitroprusside (100 μM), which acts directly on the vascular smooth muscle, was perfused at the end of each experiment (20 min after cessation of perfusion with agonist) as an internal control.

2.3. Drugs

Substance P and neurokinin A were obtained from Auspep, Melbourne, Australia. Vasoactive intestinal polypeptide was from Peninsula Laboratories, USA. Adenosine 5′-triphosphate (ATP, sodium salt), sodium nitroprusside, 5-hydroxytryptamine (serotonin) hydrochloride and N^G-nitro-L-arginine and N^G-nitro-D-arginine were from Sigma Chemical Company, USA. All drugs were dissolved in Ringer's solution.

2.4. Statistics and expression of data

Three parameters of the vasodilator response to substance P were measured: (1) the area under the response curve (flow × time; cm^2) using a digital planimeter (Tamaya, Japan), (2) the maximum height of the response (cm), (3) the time for the response to return to baseline (min). For all other agents the vasodilator response was measured as the area during 8 min of perfusion (cm^2) (this was an exact fraction of the total area, no differences being observed in onset or offset of the response in the presence of inhibitors of nitric oxide synthase). Plasma protein extravasation was measured as μg ml^{-1} and expressed as an increase above basal release. Results were expressed as mean ± S.E.M. Vasodilatation data were analysed using analy-

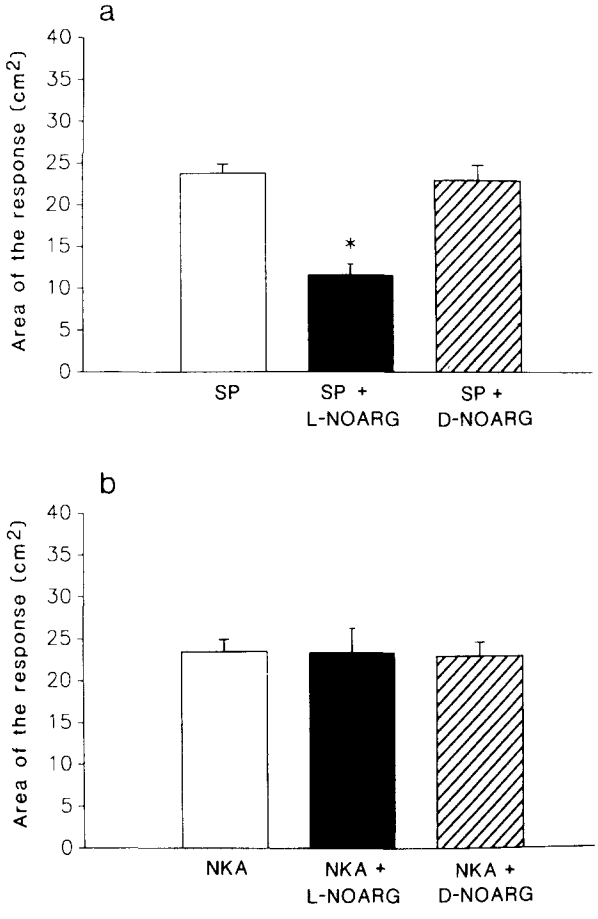

Fig. 2. Effect of N^G-nitro-L-arginine (L-NOARG) or N^G-nitro-D-arginine (D-NOARG) (both at 100 μM) on the dilatation responses of the rat skin microvasculature to (a) substance P (SP; 1 μM), (b) neurokinin A (NKA; 1 μM). (a) Substance P alone (open column, $n = 11$); substance P + N^G-nitro-L-arginine (filled column, $n = 6$); substance P + N^G-nitro-D-arginine (cross-hatched column, $n = 5$). Neurokinin A alone (open column, $n = 12$); neurokinin A + N^G-nitro-L-arginine (filled column, $n = 6$); neurokinin A + N^G-nitro-D-arginine (cross-hatched column, $n = 5$). Vertical bars indicate S.E.M. * Statistically significant difference from control.

sis of variance (ANOVA) with post-hoc Student-Newman-Keuls pairwise comparisons where necessary. Plasma extravasation data were analysed using repeated measures ANOVA with planned contrasts. Type 1 error rate was set at $\alpha = 0.05$ for all tests.

3. Results

3.1. Effect of N^G-nitro-L-arginine on vasodilator responses to substance P and neurokinin A

There was no significant effect of N^G-nitro-L-arginine and N^G-nitro-D-arginine on the baseline.

Substance P produced concentration-dependent vasodilatation in the skin microvasculature. The maximum heights of the responses were 3.28 ± 0.21 cm ($n = 5$), 5.72 ± 0.17 cm ($n = 5$) and 6.86 ± 0.19 cm ($n = 5$) at 0.1, 1 and 10 μM substance P respectively. The responses gradually declined, possibly due to desensitization; full loss of the response to 1 μM substance P occurred in 14.8 ± 1.6 min ($n = 6$) (Fig. 1). N^G-Nitro-L-arginine (100 μM) significantly attenuated the vasodilator response to substance P (Fig. 2a). This was primarily a reflection of a significant reduction in the duration of the response; full desensitization now occurred in 9.0 ± 0.7 min ($n = 6$). The maximum height of the response was not significantly different in control and N^G-nitro-L-arginine-treated rats (7.3 ± 0.7 and 4.9 ± 0.6 cm ($n = 6$) respectively). N^G-Nitro-D-arginine had no effect on vasodilatation due to substance P (Fig. 2a).

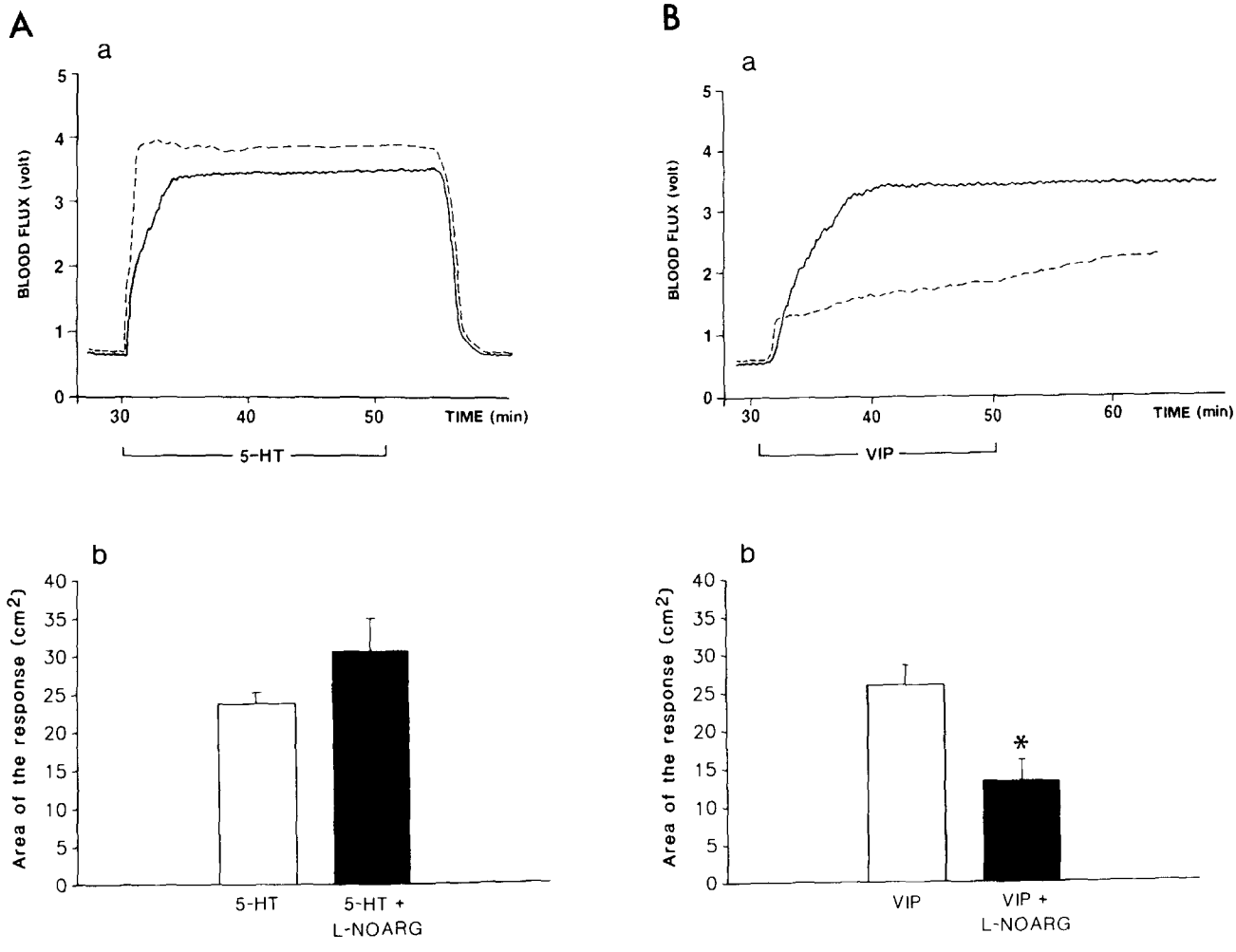

Fig. 3. Vasodilator and plasma extravasation responses to (A) 5-hydroxytryptamine (5-HT) and (B) vasoactive intestinal polypeptide (VIP) in the absence and presence of N^G-nitro-L-arginine (L-NOARG). A: (a) Representative trace showing the effect of N^G-nitro-L-arginine (100 μM) on the vasodilator response to 5-hydroxytryptamine (1 μM) perfused for 20 min over a blister base on the hind footpad of a rat. Unbroken line indicates control response; broken line indicates response in the presence of N^G-nitro-L-arginine. (b) Dilatation responses to 5-hydroxytryptamine in the absence (open column, $n = 5$) and presence (filled column, $n = 7$) of N^G-nitro-L-arginine. Vertical bars indicate S.E.M. B: (a) Representative trace showing the vasodilator response of rat skin microvasculature to vasoactive intestinal polypeptide (1 μM) perfused for 20 min in the absence and presence of N^G-nitro-L-arginine (100 μM). Unbroken line indicates control response; broken line indicates response in the presence of N^G-nitro-L-arginine. (b) Dilatation responses to vasoactive intestinal polypeptide in the absence (open column, $n = 6$) and presence (filled column, $n = 7$) of N^G-nitro-L-arginine. Vertical bars indicate S.E.M. * Statistically significant difference from control.

Neurokinin A elicited concentration-dependent vasodilatation in the rat skin microvasculature. The maximum heights of the responses were 2.18 ± 0.10 cm (n = 5), 4.26 ± 0.24 cm (n = 5) and 5.60 ± 0.32 cm (n = 5) at 0.1 μM, 1 μM and 10 μM neurokinin A respectively. At each concentration the maximum height of the response to neurokinin A was significantly less than that to substance P. Vasodilator responses to neurokinin A did not undergo desensitization being maintained as long as neurokinin A was perfused, but returned to baseline following washout. There was no significant difference in the vasodilator response to neurokinin A (1 μM) in the presence of N^G-nitro-L-arginine (Fig. 2b). N^G-Nitro-D-arginine had no effect on vasodilatation due to neurokinin A (Fig. 2b).

3.2. Effect of N^G-nitro-L-arginine on vasodilator responses to 5-hydroxytryptamine, ATP, vasoactive intestinal polypeptide and sodium nitroprusside

Vasodilator responses to 5-hydroxytryptamine (1 μM) (Fig. 3A), ATP (50 μM) and sodium nitroprusside (100 μM) were maintained as long as these agents were in contact with the blister base, but returned to baseline following washout. Vasoactive intestinal polypeptide (1 μM) elicited vasodilatation which was maintained during perfusion with this peptide and for more than 20 min thereafter, despite washout (Fig. 3B).

In the presence of N^G-nitro-L-arginine (100 μM) vasodilator responses to 5-hydroxytryptamine (Fig. 3A), ATP and sodium nitroprusside were not significantly different from those under control conditions. Area under the response curve to ATP was 40.26 ± 5.05 cm^2 (n = 6) and 38.25 ± 2.90 cm^2 (n = 6) in the absence and presence of N^G-nitro-L-arginine respectively. Responses to sodium nitroprusside were 35.01 ± 3.56 cm^2 (n = 6) and 38.88 ± 4.47 cm^2 (n = 6) in the absence and presence of N^G-nitro-L-arginine respectively. The vasodilator response to vasoactive intestinal polypeptide was significantly attenuated by N^G-nitro-L-arginine (Fig. 3B).

3.3. Plasma extravasation

Basal plasma extravasation was 43.21 ± 3.8 μg ml^{-1} (n = 27). N^G-Nitro-L-arginine and N^G-nitro-D-arginine did not significantly affect basal plasma extravasation which was 56.17 ± 7.61 μg ml^{-1} (n = 19) and 38.0 ± 1.55 μg ml^{-1} (n = 10) in the presence of these agents respectively.

3.4. Effect of N^G-nitro-L-arginine on plasma extravasation due to substance P and neurokinin A

Substance P (1 μM) produced an increase in plasma extravasation during the 30 min of perfusion and for more than 20 min thereafter. In the presence of N^G-nitro-L-arginine (100 μM) substance P-induced plasma extravasation was significantly attenuated (Fig. 4a). N^G-Nitro-D-arginine had no effect on substance P-induced plasma extravasation (Fig. 4b). Neurokinin A (1 μM) elicited an increase in plasma extravasation which was not significantly affected by N^G-nitro-L-arginine (Fig. 5a) or N^G-nitro-D-arginine (Fig. 5b). The difference in plasma extravasation due to substance P alone in two groups of animals represented by data in Fig. 4a and Fig. 4b cannot be explained, however, since the experiments with N^G-nitro-L-arginine and N^G-nitro-D-arginine were run as independently controlled groups

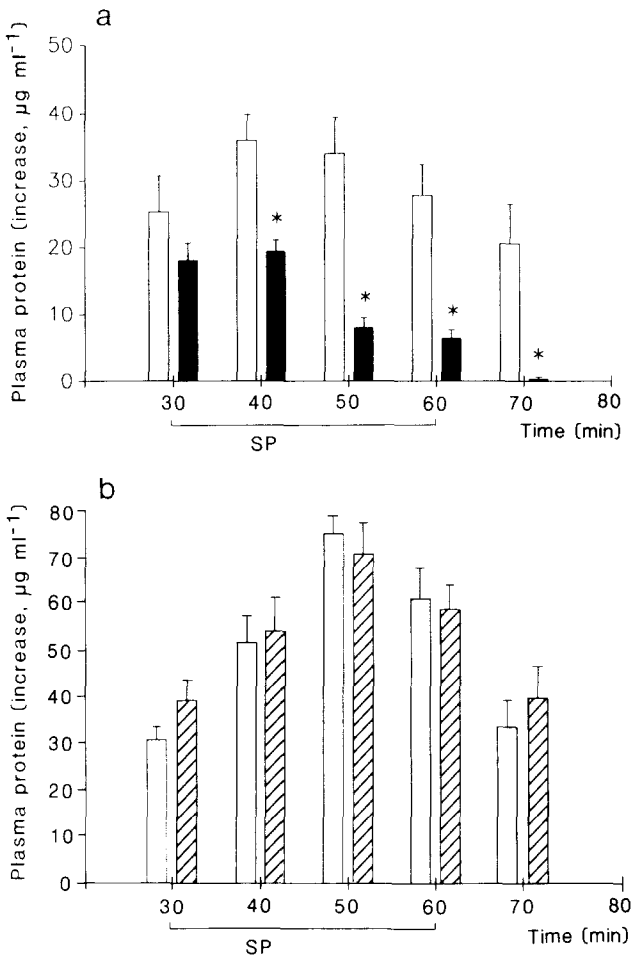

Fig. 4. Effect of (a) N^G-nitro-L-arginine or (b) N^G-nitro-D-arginine (both at 100 μM) on plasma extravasation response to substance P (SP, 1 μM superfused for 30 min (horizontal line) after an initial 30 min of equilibration). Columns indicate plasma protein content (increase above basal, μg ml^{-1}) of consecutive 10 min fractions, with time of onset of collection indicated beneath columns. (a) Substance P alone (open columns, n = 6); substance P + N^G-nitro-L-arginine (filled columns, n = 6). (b) Substance P alone (open columns, n = 5); substance P + N^G-nitro-D-arginine (cross-hatched columns, n = 5). Vertical bars indicate S.E.M. *Statistically significant difference from control.

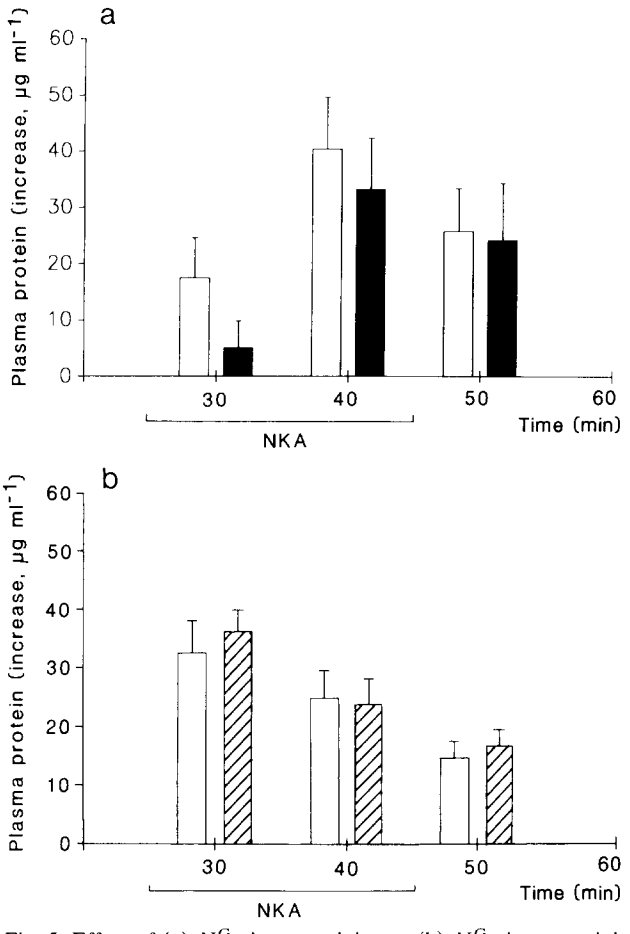

Fig. 5. Effect of (a) N^G-nitro-L-arginine or (b) N^G-nitro-D-arginine (both at 100 μM) on plasma extravasation response to neurokinin A (NKA, 1 μM superfused for 20 min (horizontal line) after an initial 30 min of equilibration). Columns indicate plasma protein content (increase above basal, μg ml^{-1}) of consecutive 10 min fractions, with time of onset of collection indicated beneath columns. (a) Neurokinin A alone (open columns, $n = 7$); neurokinin A + N^G-nitro-L-arginine (filled columns, $n = 7$). (b) Neurokinin A alone (open columns, $n = 5$); neurokinin A + N^G-nitro-D-arginine (cross-hatched columns, $n = 5$). Vertical bars indicate S.E.M. In a second post-stimulation fraction (at time 60 min) there was no increase above basal plasma extravasation.

a difference does not invalidate any of the conclusions drawn.

3.5. Effect of N^G-nitro-L-arginine on plasma extravasation due to 5-hydroxytryptamine

5-Hydroxytryptamine (1 μM) produced a significant increase in plasma extravasation which was not affected by N^G-nitro-L-arginine. The increase in plasma extravasation above basal in four consecutive fractions (each of 10 min) was 22.46 ± 5.02, 46.59 ± 4.22, 45.02 ± 2.64 and 11.72 ± 2.35 μg ml^{-1} ($n = 4$) in the absence of N^G-nitro-L-arginine, and 24.49 ± 7.53, 42.39 ± 5.52, 37.95 ± 9.05 and 10.45 ± 8.16 μg ml^{-1} ($n = 6$) in the presence of N^G-nitro-L-arginine.

ATP, vasoactive intestinal polypeptide and sodium nitroprusside did not elicit plasma extravasation.

4. Discussion

In large blood vessels vasodilatation due to substance P and other tachykinins appears to be mediated exclusively by activation of tachykinin NK_1 receptors located on vascular endothelial cells with subsequent generation of endothelium-derived relaxing factor or nitric oxide (D'Orleans-Juste et al., 1986; Regoli et al., 1988). The tachykinin NK_1 receptor has also been implicated in neurokinin-induced microvascular dilatation and increases in capillary permeability (Andrews et al., 1989; Jacques et al., 1989; Lam and Ferrell, 1993; Hirayama et al., 1993). In the microcirculation activation of the NK_1 receptor is known to involve hydrolysis of inositol phospholipids (Thomas et al., 1989); however, the role of nitric oxide in these responses is less well-defined. Our results demonstrate that in rat skin microvasculature, nitric oxide acts as a second messenger to substance P-induced inflammatory responses since N^G-nitro-L-arginine, a selective inhibitor of nitric oxide biosynthesis, attenuated substance P-induced vasodilatation and plasma extravasation. The action of N^G-nitro-L-arginine was selective in that it did not affect vasodilatation due to sodium nitroprusside, which acts directly on the vascular smooth muscle. Furthermore, N^G-nitro-D-arginine, the inactive isomer of N^G-nitro-L-arginine, had no effect on vasodilatation or plasma extravasation due to substance P or neurokinin A. The lack of effect of N^G-nitro-L-arginine on baseline responses suggests that there is no significant basal release of nitric oxide in rat skin microvasculature.

In the presence of N^G-nitro-L-arginine, inhibition of the vasodilator response to substance P was manifested primarily as an attenuation of the duration rather than the amplitude of the response. In a previous study, using the same rat skin model, we found that N^G-nitro-L-arginine and N^G-monomethyl-L-arginine also preferentially inhibited the duration of the vasodilator response to acetylcholine, which acts via endothelium-dependent mechanisms in vitro and in vivo and also undergoes tachyphylaxis in our system (Ralevic et al., 1992b). The mechanisms involved in tachyphylaxis are complex, however, since a return to baseline for both substance P and acetylcholine occurs more readily in the presence of N^G-nitro-L-arginine, this suggests that nitric oxide may be implicated in maintaining such responses.

In the microvasculature, while vasodilatation is primarily a function of arterioles, plasma extravasation is primarily a function of the post-capillary venules (Kenins et al., 1984). These events are largely indepen-

dent since agents such as sodium nitroprusside and ATP which elicit vasodilatation do not also cause plasma extravasation. In addition, there appeared to be no direct correlation between the time course of substance P-induced vasodilatation and plasma extravasation. Hence, it is possible that N^G-nitro-L-arginine attenuated substance P-induced plasma extravasation via a direct inhibition of nitric oxide derived from the endothelium of postcapillary venules. Consistent with this, N^G-nitro-L-arginine methyl ester inhibited oedema formation induced by substance P in rat skin in a model in which the ability of substance P to increase local blood flow was weak (Hughes et al., 1990). On the other hand, since there is evidence that vasodilatation and plasma extravasation are related, attenuation of plasma extravasation by N^G-nitro-L-arginine may have partly been a reflection of inhibition of vasodilatation occurring at the level of precapillary arterioles. Consistent with the hypothesis that events occurring at the level of arterioles can influence events at the level of venules is the observation that vasodilators which do not themselves increase vascular permeability may enhance plasma extravasation to agents which do (Brain and Williams, 1985).

Concentration-response curves showed substance P to be a more potent vasodilator of the skin microvasculature than neurokinin A, as also found in human skin (Fuller et al., 1987; Wallengren and Håkanson, 1987). However, it should be noted that this comparison was based on the maximal heights of the responses, not taking into account the fact that responses to neurokinin A are not subject to the marked tachyphylaxis that was characteristic of vasodilatation to substance P. Vasodilator and plasma extravasation responses due to neurokinin A, unlike those to substance P, were not inhibited by N^G-nitro-L-arginine. This may be related to the different abilities of the two tachykinins to activate mast cells. It is known that rat mast cells possess only tachykinin NK_1 receptors and that stimulation of rat mast cells by substance P is effected via its N-terminal (Devillier et al., 1985). Since neurokinin A did not undergo tachyphylaxis it is likely that its vasodilator and plasma extravasation responses in rat skin are mediated via the tachykinin NK_2 receptor. While neurokinin A possesses C-terminal homology with substance P, it lacks the N-terminal necessary for activation of mast cells. Hence, it is possible that in our system the component of the vasodilator and plasma extravasation response to substance P which is inhibited by N^G-nitro-L-arginine is that resulting from an action of nitric oxide derived from mast cells (Salvemini et al., 1990). Therefore, while the endothelium is the most likely source of nitric oxide we cannot exclude the possible contribution of mast cells as a source of nitric oxide. There may be species differences in this effect since both substance P and neurokinin A evoke histamine release primarily via a tachykinin NK_2 receptor in a cloned mouse mast cell line (Krumins and Broomfield, 1992).

Abundant vasoactive intestinal polypeptide-containing nerve fibres are present in the skin of rat and other species where they may be associated with the cutaneous vasodilatation that accompanies sweating, as well as with the inflammatory response (Hughes and Brain, 1991; Smith et al., 1992). In isolated large blood vessels vasodilatations to vasoactive intestinal polypeptide both in the presence and absence of the endothelium have been described, and in vessels of the cat submandibular gland (Edwards and Garrett, 1993) and human gastroepiploic and internal mammary arteries (Luu et al., 1993) nitric oxide was implicated in the endothelium-dependent effect. At the level of the microvasculature the neural-endothelial separation is small, raising the possibility of interactions between substances released from perivascular nerves and the microvascular endothelium. The results of the present study show, for the first time, a role for the endothelium and nitric oxide in vasoactive intestinal polypeptide-mediated relaxation in rat skin microvasculature.

Vasodilatation due to 5-hydroxytryptamine and ATP in isolated large blood vessels also generally proceeds via activation of receptors located on endothelial cells and subsequent release of nitric oxide (Furchgott, 1983; Rees et al., 1989). Responses to the endothelium-dependent vasodilators A23187 (Ca^{2+} ionophore; calcimycin) and acetylcholine are inhibited by both N^G-nitro-L-arginine and N^G-monomethyl-L-arginine in rat skin microvasculature (Ralevic et al., 1992b). However, N^G-nitro-L-arginine did not inhibit vasodilator responses due to neurokinin A, 5-hydroxytryptamine or ATP or plasma extravasation due to neurokinin A or 5-hydroxytryptamine. Another selective inhibitor of nitric oxide biosynthesis and of endothelium-dependent relaxations in vitro, N^G-monomethyl-L-arginine, also did not inhibit these responses (unpublished observations). A lack of inhibition by N^G-nitro-L-arginine or N^G-nitro-L-arginine methyl ester of responses due to endothelium-dependent vasodilators has previously been observed in vivo (Aisaka et al., 1989; Gardiner et al., 1990). Several possibilites for this have been discussed by Gardiner et al. (1990) and include: (1) the presence of a preformed pool of nitric oxide; (2) efficient receptor-effector coupling; and (3) these agents may act through mechanisms independent of nitric oxide. These and additional possibilities are likely in our system since the inflammatory response is complex involving several components including endothelial cells, sensory nerves and mast cells.

Substance P, neurokinin A and ATP can release prostacyclin from cultured endothelial cells which may contribute to nitric oxide-independent vasodilator mechanisms (Marceau et al., 1989; Gordon, 1986). Sen-

sory nerve endings are activated and sensitized by 5-hydroxytryptamine (Birrell et al., 1990; Khalil and Helme, 1990) and it has been estimated that about 50% of the vasodilator response of rat skin microvasculature to 5-hydroxytryptamine is mediated via an action on sensory nerves (Khalil and Helme, 1990). Hence, the release of nitric oxide-independent vasodilator agents from sensory nerves (or from other nerves) could also contribute to vasodilatation in our system. Mast cells are activated by substance P, vasoactive intestinal polypeptide, A23187 and ATP to release mediators of inflammation such as histamine and prostaglandins (Church et al., 1989; Jaffar and Pearce, 1990; West, 1990) and it is possible that these and other mast cell products may contribute to nitric oxide-independent mechanisms of dilatation. Hyperpolarization may also be involved (Beny and Brunet, 1988; Chen and Suzuki, 1991). Finally, it is possible that vasodilatation may be effected via a direct action on the vascular smooth muscle. In some vessels P_{2Y} purinoceptors mediating vasodilatation to ATP are located on the vascular smooth muscle (Ralevic and Burnstock, 1991). Endothelium-independent relaxation has also been demonstrated for 5-hydroxytryptamine in small arterioles of skeletal muscle (Alsip et al., 1987).

In conclusion, we have shown that nitric oxide is implicated in neurogenic inflammatory mechanisms in rat skin microvasculature since it is the mediator of a component of the vasodilator and plasma extravasation response to substance P, and of the vasodilator response to vasoactive intestinal polypeptide. The most likely source of this nitric oxide is the vascular endothelium, although we cannot exclude a contribution of nitric oxide derived from mast cells. Inflammatory responses to neurokinin A, 5-hydroxytryptamine and ATP proceed via mechanisms which appear to be independent of nitric oxide. This, and the presence of a N^G-nitro-L-arginine-resistant component of responses due to substance P and vasoactive intestinal polypeptide suggest that the microvasculature may differ substantially from large vessels in its response to vasoactive substances.

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