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L-Arginine and N^G-nitro-L-arginine methyl ester cause macromolecule extravasation in the microcirculation of awake hamsters

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Abstract

We investigated the effects of L-arginine and N^G-nitro-L-arginine methyl ester (L-NAME) on macromolecule extravasation in the microcirculation of awake hamsters by computer-assisted image analysis of the distribution of FITC (fluorescein isothiocyanate)-dextran fluorescence in dorsal fold skin preparations. This analysis made it possible to simultaneously study the time course of local (skin) and general (all irrigated organs) extravasation in 180-min experiments. Bolus injection of 30 or 150 mg/kg (i.v.) L-arginine induced immediate local and general macromolecule leakage and delayed venule dilation beginning 1 h later. Injection of 20 or 100 mg/kg (i.v.) L-NAME caused rapid venule constriction followed by local and general extravasation beginning 45–60 min later. These effects of L-arginine and L-NAME were not mimicked by their biologically inactive isomers, D-arginine and D-NAME. Simultaneous bolus injection of 20 mg/kg L-NAME and 150 mg/kg L-arginine caused no significant change in fluorescence distribution or venule diameter. L-arginine effects on macromolecule extravasation were mimicked by sodium nitroprusside (10 μg/kg, i.v.) and by 8-bromo-cGMP (1 mg/kg, i.v.). Sodium nitroprusside was ineffective on venule diameter. The effects of both L-arginine and sodium nitroprusside on FITC-dextran extravasation were prevented by simultaneous injection (10 μg/kg, i.v.) of the specific inhibitor of the soluble guanylate cyclase, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). This dose of ODQ mimicked the effects of L-NAME on macromolecule extravasation and venule diameter. Taken together, these results suggest that activation or inhibition of basal NO synthesis might induce macromolecule leakage in the microcirculation of awake hamsters via temporally distinct cGMP-dependent mechanisms. © 1998 Elsevier Science B.V.

Keywords: Microcirculation; Nitric oxide (NO); Nitric oxide (NO) synthase; Permeability

1. Introduction

Nitric oxide (NO) is synthetized from L-arginine by the constitutive Ca^{2+}-dependent NO synthase of endothelial cells. In addition to its vasodilator activity, endothelium-derived NO influences microvascular permeability. Studies of the effects of NO synthesis on macromolecule leakage have not produced consistent results (Kubes, 1995). NO synthesis has been shown both to prevent and cause macromolecule leakage from the microvasculature depending on the experimental system and conditions used. In vivo, the rapid and transient macromolecule leakage induced by superfusion of inflammatory mediators such as histamine on exteriorized organs in anaesthetized animals is due to stimulation of the endothelial NO synthase (Yuan et al., 1993; Mayhan, 1994). The subsequent rise in cGMP concentration causes the formation of gaps between endothelial cells (Yuan et al., 1993; Hölschermann et al., 1997). Consistent with these in vivo studies, inhibition of endothelial NO synthase by N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA) decreases baseline and prevents the flow-dependent increase in the permeability of isolated coronary venules (Yuan et al., 1992). Thus, acute stimulation of the constitutive endothelial NO synthase by inflammatory mediators or blood flow promotes rapid leakage of macromolecules by a cGMP-dependent mechanism. Numerous in vivo and in vitro studies have shown that inhibition of NO synthase provokes macromolecule leakage. Inhibition of NO syn-

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thase by analogues of L-arginine increases leukocyte adherence and migration (Kubes et al., 1991; Davenpeck et al., 1994; Suematsu et al., 1994; Kurose et al., 1995; Niu et al., 1996). Because leukocyte migration is closely associated with increased microvascular permeability (Kubes and Gaboury, 1996), inhibition of basal NO production causes delayed leukocyte-dependent extravasation (Kubes and Granger, 1992).

None of the previous reports dealing with L-arginine and analogues has studied the role of basal NO synthesis on endothelial barrier function in the microcirculation of awake animals. In this study, using quiescent hamster dorsal fold skin preparations (Endrich et al., 1980) and intravital microscopy, we investigated the effects of NO synthase inhibition or activation on FITC (fluorescein isothiocyanate)-dextran leakage.

2. Materials and methods

2.1. Drugs

FITC-dextran (150 000 MW), N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME), L-arginine, D-arginine and sodium nitroprusside were dissolved in saline (NaCl 0.9%). 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was dissolved in DMSO/NaCl 0.9% (1:1000 v/v). ODQ was purchased from Calbiochem (Meudon, France) and all other drugs from Sigma Chemical (Saint-Quentin Fallavier, France).

2.2. Surgical procedure

A titanium window chamber that allows observation of the microcirculation in the skin of the back was implanted in anaesthetized (pentobarbital, 60 mg/kg, i.p.) male Syrian golden hamsters (weight 60–80 g) as described by Endrich et al. (1980). Briefly, the back of the anaesthetized hamster was shaved and a depilatory ointment was applied to remove any remaining hair. Two titanium frames were sewn so as to sandwich the extended double layer of dorsal skin. Once the first frame of the chamber was fixed, the opposite skin layer was completely removed in a defined circular area (15 mm diameter). The remaining exposed layer was moistened with 0.9% saline solution and covered by a cover slip fixed onto the second frame, thereby allowing observation of the microcirculation through the chamber. A subcutaneous venous catheter was inserted into the jugular vein, positioned at the dorsal side of the neck and sutured to the frames. The animal was allowed to recover from anaesthesia and surgery for 48 h before the experiments. The dorsal skin preparation was quiescent with respect to the endothelial barrier to macromolecules as no inflammation was observed in the striated muscle tissue contained in the chamber, as assessed by both optical and electron microscopy (Endrich et al., 1980).

2.3. Experimental set-up and computer-assisted image analysis

Experiments were performed two days after the surgical procedure in a dark, quiet, and temperature-controlled (21°C) laboratory. The awake animal was placed in a specially designed cylinder which, once fixed on the microscope, allowed observation of the skin microcirculation at a magnification of $\times 125$ throughout the 180-min long experiments. The fluorescent macromolecular tracer, FITC-dextran 150 kDa (FD-150; 63 mg/kg), was injected via the jugular catheter and allowed to equilibrate for 15 min. The preparation was then epi-illuminated at the optimal excitation wavelength for FITC (450-490 nm), using a 100-W mercury bulb with filters (I3 blue block, Leica) positioned between the light source and the condenser. A CCD black and white video camera (HPR 610, Instrumentation Capteur, Meylan, France) was connected to the microscope and images were sent to a VHS video-recorder for off-line computer-assisted image analysis.

Epi-illumination of the preparation after equilibration of FD-150 fluorescence allowed visualisation of the venules and arterioles of the skin microcirculation (Fig. 1). The dorsal skin preparation was sequentially illuminated (for 1 min, at intervals of 15 min) from $t = 0$ min (1 min before bolus injection of the test molecules) until the end of the experiments (time $t = 180$ min). Analogue video images were collected every 15 min and were digitized into x, y arrays of 512 by 512 pixels (Visicap software) as previously described (Bekker et al., 1989). With this digitization procedure, each pixel was associated with an 8-bit grey level (a number between 0 (black) and 255 (white)). The grey scale histogram was constructed by scanning the entire digitized image. Fitting 2 or 3 Gaussian curves (Microcal Origin software) to this histogram showed that pixels were normally distributed in two main homogeneous populations along the grey scale axis. At $t = 0$ (i.e., 1 min before bolus injection of test molecules), these peaks were centred at 81 ± 7 (35 ± 3% of the total population of pixels) and 177 ± 6 (53 ± 5% of the total population of pixels) ($n = 130$) of the 256 grey levels. The right-hand peak resulted from the brighter venule population of pixels whereas the left-hand peak accounted for the darker interstitial population of pixels. Arterioles were always darker than venules and were responsible for a third minor peak. The difference between arteriole and venule grey levels may be due to the three-dimensional nature of the preparation and to the smaller diameter of arterioles.

2.4. Measurements of drug-induced macromolecule extravasation

Injection of saline solution (at $t = 1$ min) caused no significant change in venules and interstitial fluorescence during 3-h experiments. Consequently, the grey level histograms were similar at $t = 0$ min (Fig. 1A, top) and

$t = 180$ min (Fig. 1A, bottom). After 3 h injection of L-arginine (Fig. 1B, bottom) or L-NAME (Fig. 1C, bottom), interstitial fluorescence was higher and venule fluorescence was lower than those measured at $t = 0$ min. Changes in interstitial fluorescence were assumed to account for FD-150 accumulation in the skin (local extravasation). Changes in venule fluorescence were assumed to account for loss of FD-150 by the vasculature in all organs (general extravasation) since L-arginine and L-NAME were systemically applied. L-arginine and L-NAME-induced fluorescence redistribution accounted for a rightward shift of the interstitial peak and for a leftward shift of the venule peak in the grey level histograms (Fig. 1B,C; bottom). Throughout the 180-min experiments, the amplitudes of the peak shifts (X_1 and X_2), measured between time t and $t = 0$, were determined every 15 min. They were assumed to be proportional to the amount of FD-150 accumulated in the skin (X_1) and to the amount of FD-150 lost by the vasculature in all organs (X_2). Because the extent of macromolecule diffusion through the permeabilized endothelium depends on the pre-existing gradient of concentrations at $t = 0$ min, both shifts were normalized by using the difference between venule and interstitial mean grey levels at $t = 0$ (X_T). Therefore, 'local extravasation' was expressed as X_1/X_T and 'general extravasation' was expressed as X_2/X_T.

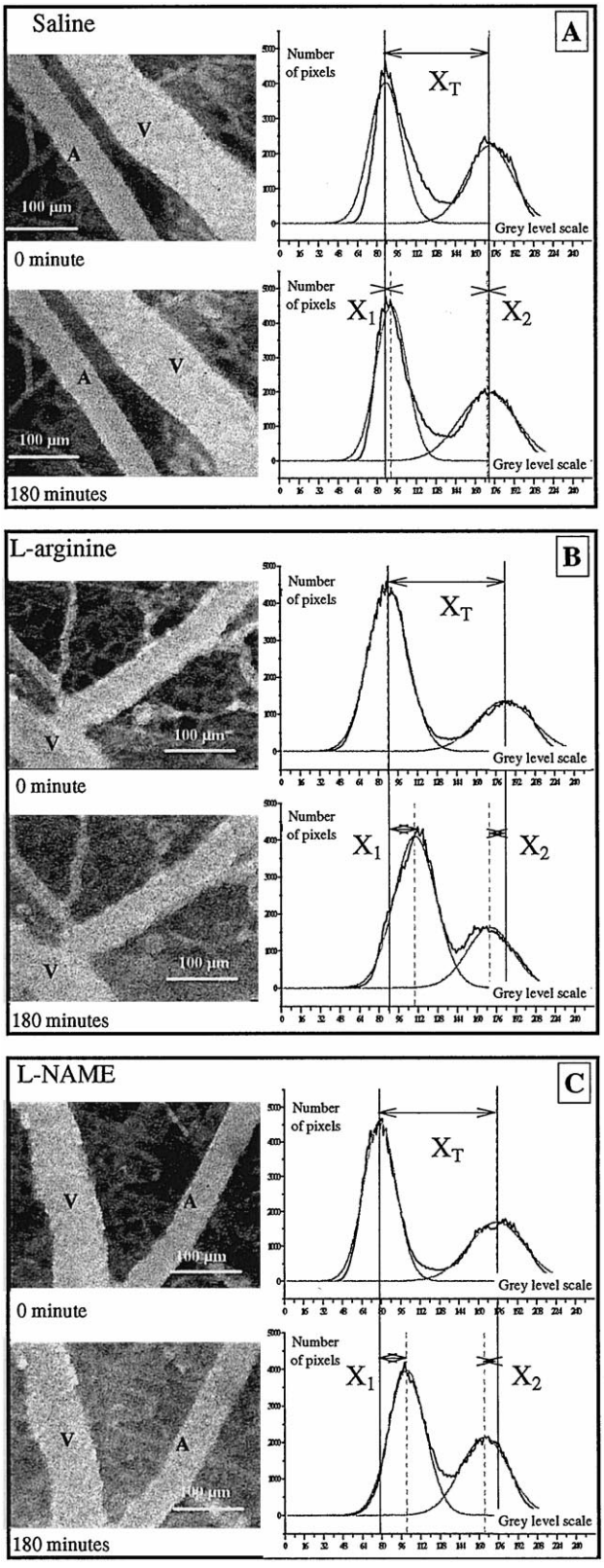

Fig. 1. Effects of intravenous administration ($t = 1$ min) of saline solution (0.9% NaCl), L-arginine (150 mg/kg) or L-NAME (100 mg/kg) on fluorescence distribution between vascular (A = arteriole; V = venule) and interstitial compartments in dorsal fold skin preparation of awake hamster. Images were collected from representative 180-min experiments at the start ($t = 0$ min) and the end of the experiments ($t = 180$ min). The corresponding grey scale level histograms were derived from computer-assisted image analysis fitted with the Microcal Origin Peak Fitting Module software. The time-dependent increase in interstitial light intensity (left-hand peak) resulted in a rightward shift of the mean grey level of the peak corresponding to the darker pixels (X_1). Intravenule light intensity (right-hand peak) decreased, resulting in a leftward shift of the mean grey level of the peak corresponding to the brighter pixels (X_2). X_1 referred as local extravasation index and X_2 referred as general extravasation index were normalized with X_T (before intravenous administration).

2.5. Venule diameter measurements

The diameters of venules and arterioles were also measured from the digitized images because pharmacological interventions that stimulate or inhibit NO synthesis exert profound effects on haemodynamic factors. Unfortunately, due to the vasomotion observed in awake animals, the effects of the molecules on arteriole diameter were not consistent. Thus, changes in venule diameter were used as an index of the overall cardiovascular effects of the molecule tested. Changes observed in venule diameter did not necessarily reflect a direct action of the molecule on the venule wall and could result from upstream effects (Messina et al., 1975; Warren, 1994).

To measure venule diameter on each image, a grey level threshold was defined between the interstitial and

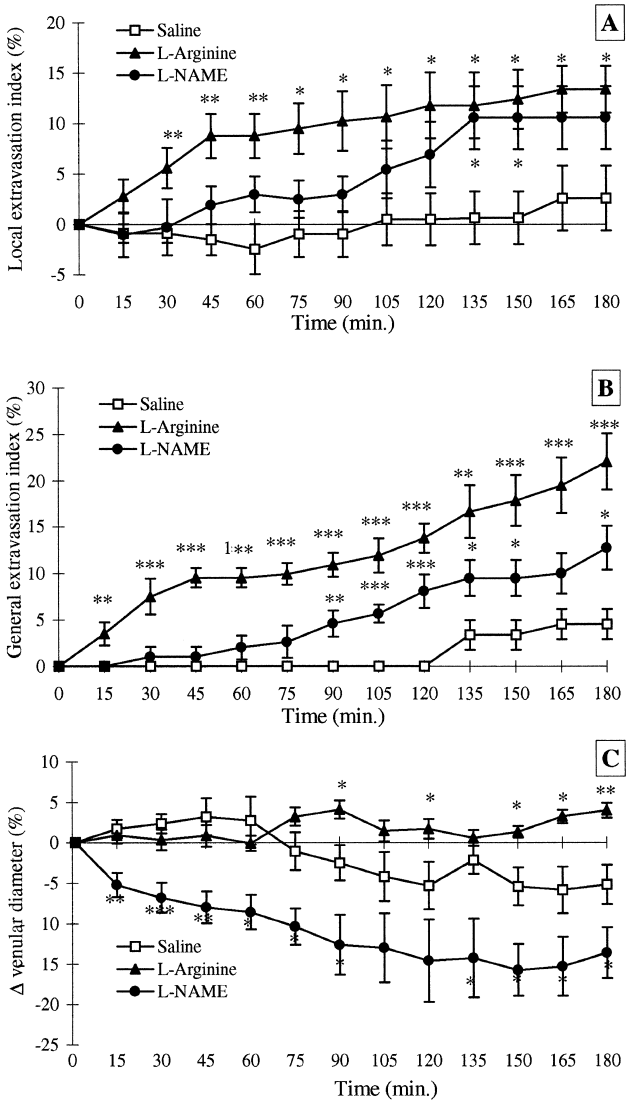

Fig. 2. Time-course of local (A) and general (B) extravasation and changes in venule diameter (2C) induced by the intravenous administration of saline solution (0.9% NaCl), L-arginine (150 mg/kg) or L-NAME (100 mg/kg). ∗Denotes a significant difference with the time point obtained after saline injection according to Scheffe's post hoc statistical test (∗ $P < 0.05$; ∗∗ $P < 0.01$; ∗∗∗ $P < 0.001$).

venule grey levels. Pixels with values higher than this threshold were automatically changed to black (grey level 0), and those lower than this threshold to white (grey level 255). Thus, the vascular compartment was shown in black and interstitial compartment in white. A segment was defined perpendicular to the longitudinal axis of the vessel. Visicap software determined the number of black pixels along this segment and converted it into microns. The collecting venules selected in this study had an initial diameter of 93 ± 10 μm. Drug-induced changes in venule diameter were measured every 15 min and expressed as a percentage of the initial diameter measured at $t = 0$ min (before drug administration).

2.6. Mean arterial pressure measurements

We were unable to measure arterial blood pressure in awake hamsters. Indeed, we found that clots rapidly collapsed catheters inserted in the left carotid artery during window chamber implantation. Therefore, male Syrian golden hamster (12–14 week old; weight 120–150 g) were anaesthetized with pentobarbital (60 mg/kg, i.p.). The left carotid artery (PE-10, Clay Adams, NJ, USA) was cannulated for the measurement of mean arterial blood pressure while the right jugular vein (S54-HL, Bioblock, France) was cannulated for systemic administration of drugs. Anaesthesia was maintained by bolus administration of pentobarbital. Drug-induced changes in mean arterial blood pressure were measured every 15 min. Mean arterial blood pressure measured before drug administration was taken as a reference.

2.7. Statistical analysis

Results are expressed as means ± S.E.M.. Analysis of variance (ANOVA) was used for statistical comparison and significance was assessed by using Scheffe's post hoc test. $P < 0.05$ was considered significant.

3. Results

The time course analysis of fluorescence changes induced by bolus injection of saline, L-arginine or L-NAME is shown in Fig. 2. Saline solution injection did not cause any FD-150 extravasation. The basal equilibrium reached 15 min after injection of the fluorescent tracer was stable throughout the 3-h experiments, suggesting that the dorsal skin preparation could effectively be assumed to be quiescent with respect to endothelial barrier function. A slight but insignificant reduction in venule diameter was observed during these control experiments.

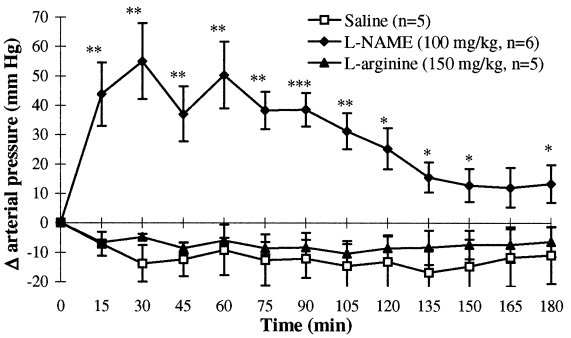

Fig. 3. Effects of intravenous administration of saline solution (0.9% NaCl), L-arginine (150 mg/kg) or L-NAME (100 mg/kg) on mean arterial blood pressure of anaesthetized hamster. The results are expressed as means ± S.E.M. ∗Indicates a significant difference (∗ $P < 0.05$; ∗∗ $P < 0.01$; ∗∗∗ $P < 0.001$; Scheffe's post hoc test) vs. saline group.

Both L-arginine (150 mg/kg, i.v.) and L-NAME (100 mg/kg, i.v.) induced local and general extravasation. However, the pro-inflammatory effects of these two compounds could be temporally and quantitatively distinguished (Fig. 2A and B). L-arginine caused immediate FD-150 accumulation in the interstitial fluid and concomitant loss of fluorescent tracer by the vasculature, whereas the effects of L-NAME on local and general extravasation developed after about 1 h. At $t = 180$ min, the effects of L-NAME on local and general extravasation were smaller than those of L-arginine. L-arginine and L-NAME affected venule diameter in opposite manners. A significant venule dilation developed 75 to 90 min after L-arginine bolus injection. An immediate reduction of the venule diameter was observed in response to L-NAME. Therefore, L-arginine-induced macromolecule leakage preceded the L-arginine-induced increase in venule diameter whereas the L-NAME-induced decrease in venule diameter preceded L-NAME-induced extravasation. Consistent with the time course of the L-NAME-induced reduction of venule diameter in awake hamsters, bolus injection of 100 mg/kg L-NAME produced a rapid and sustained rise in mean arterial pressure in anaesthetized animals (Fig. 3). L-arginine (150 mg/kg, i.v.) and saline did not modify mean arterial pressure during the 180-min experiments.

To investigate whether the effects of L-arginine and L-NAME on macromolecule leakage and venule diameter measured at the end of the experiments were due to activation and inhibition of the constitutive NO synthesis, respectively, their biologically inactive isomers were tested. Furthermore, different doses of L-arginine and L-NAME were injected alone or in combination to check their

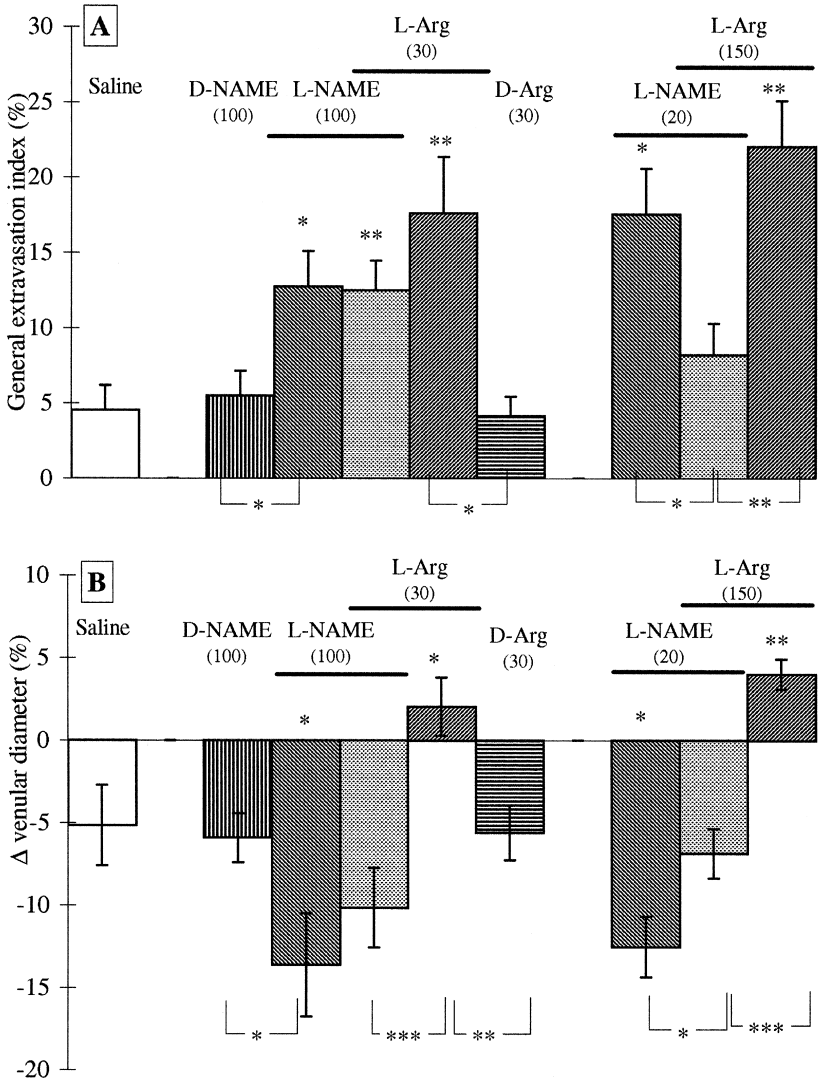

Fig. 4. Effects observed 180 min after intravenous administration of saline solution (0.9% NaCl), L-arginine (30 or 150 mg/kg), D-arginine (30 mg/kg), L-NAME (20 or 100 mg/kg) or D-NAME (100 mg/kg), given alone or in combination, on general extravasation (A) and venule diameter (B). The results are expressed as means ± S.E.M ($n = 5$–8). * Indicates a significant difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; Scheffe's post hoc test) vs. saline group or appropriate control as indicated.

reciprocal reversal effects. D-NAME (100 mg/kg, i.v.) did not cause the extravasation and reduction in venule diameter observed with the same dose of L-NAME (Fig. 4). Injection of 30 mg/kg (i.v.) D-arginine did not reproduce the effects of 30 mg/kg L-arginine. Simultaneous bolus injection of 100 mg/kg L-NAME and 30 mg/kg L-arginine produced the same final effects as were obtained in response to a single bolus injection of L-NAME. Analysis of the time course revealed that simultaneous bolus injection of 100 mg/kg L-NAME and 30 mg/kg L-arginine induced a rapid (within 15 min) reduction in venule diameter but delayed local and general extravasation (data not shown). This suggests that for this L-arginine to L-NAME ratio of 0.33, L-arginine failed to reverse the effects of L-NAME on venule diameter and on macromolecule permeability.

With 20 mg/kg L-NAME alone, the results were temporally and quantitatively similar to those obtained with 100 mg/kg (i.v.), and 150 mg/kg L-arginine caused macromolecule extravasation and an increase in venule diameter similar to those observed with 30 mg/kg. Interestingly, simultaneous bolus injection of 20 mg/kg L-NAME and 150 mg/kg L-arginine did not affect fluorescence distribution or venule diameter during the 180 min of the experiments. Thus, with an L-arginine to L-NAME ratio of 7.5, the reciprocal reversal effects of L-arginine and L-NAME could be revealed.

An exogenous NO donor, sodium nitroprusside (10 μg/kg, i.v.), and a cell-permeable analogue of cGMP, 8-bromo-cGMP (1 mg/kg, i.v.), had the same effects on macromolecule extravasation as L-arginine (Fig. 5). However, these compounds did not cause the delayed increase in venule diameter induced by the NO synthase substrate. Inhibition of guanylate cyclase activity by the specific inhibitor ODQ (10 μg/kg, i.v.) (Garthwaite et al., 1995)

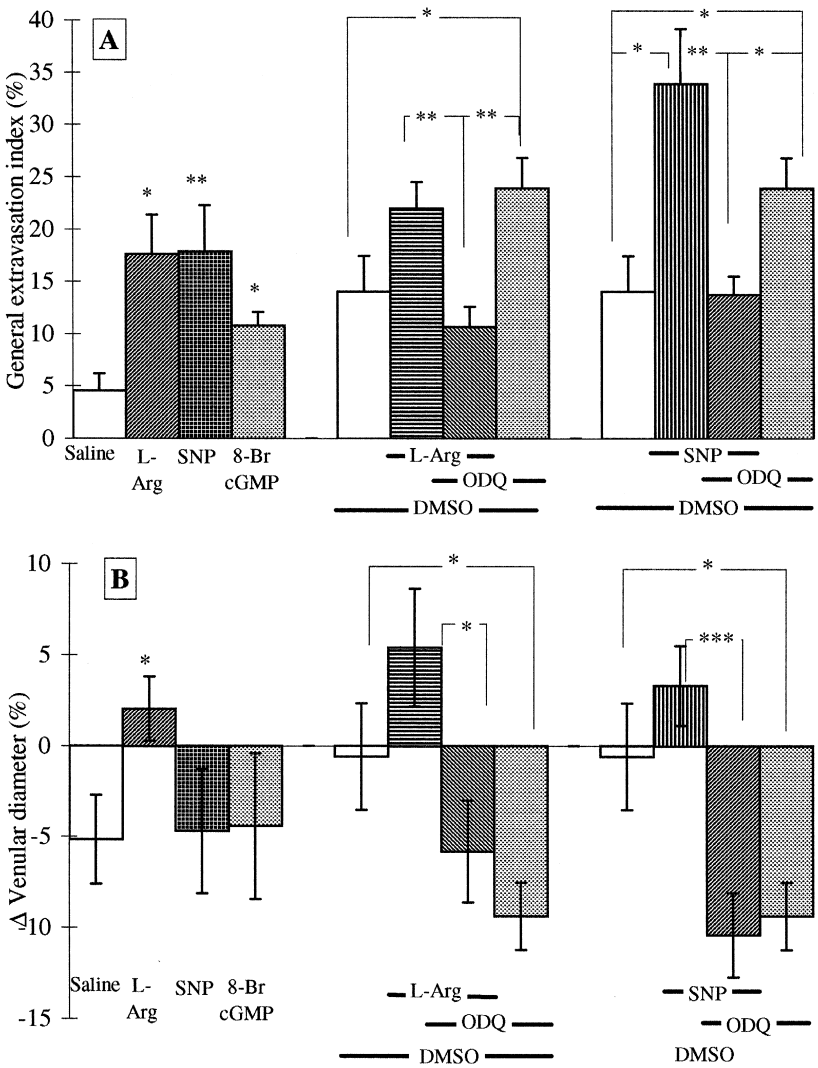

Fig. 5. Effects observed 180 min after intravenous administration of saline (0.9% NaCl), L-arginine (30 mg/kg), sodium nitroprusside (10 μg/kg), 8-bromo cGMP (1 mg/kg), DMSO (1:1000, vol:vol) or ODQ (10 μg/kg), given alone or in combination, on general extravasation (A) and venule diameter (B). The results are expressed as means ± S.E.M ($n = 5$–9). * Indicates a significant difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; Scheffe's post hoc test) vs. saline group or appropriate control as indicated.

mimicked the effects of L-NAME on macromolecule extravasation and venule diameter. Another known but less specific inhibitor of guanylate cyclase, LY 83583 (1 μg/kg, i.v.), caused some non-significant macromolecule leakage and vasoconstriction (data not shown).

Concomitant injections of L-arginine (30 mg/kg, i.v.) and ODQ (10 μg/kg, i.v.) or sodium nitroprusside (10 μg/kg, i.v.) and ODQ (10 μg/kg, i.v.) did not have a significant effect on general extravasation or venule diameter, suggesting a reciprocal reverse effect of L-arginine or sodium nitroprusside and ODQ.

4. Discussion

This study demonstrates that the permeability to macromolecules of the microcirculation of awake hamster depends on a complex balance between the protective and deleterious effects of nitric oxide. Both inhibition and activation of constitutive NO synthesis might cause macromolecule extravasation, probably through opposing cGMP-dependent mechanisms.

Intravenous administration of L-NAME caused macromolecule extravasation and a reduction in venule diameter which could be prevented by simultaneous injection of large amounts of L-arginine according to the competitive interaction of L-arginine and L-NAME with NO synthase. Inhibition of constitutive NO synthesis as a mechanism for the effects of L-NAME is supported by the lack of effect of D-NAME. Specific inhibition of the soluble form of guanylate cyclase by ODQ (Garthwaite et al., 1995) mimicked the effects of NO synthase inhibition on macromolecule permeability and on venule diameter. As for L-NAME, the ODQ-induced macromolecule extravasation and increase in venule diameter could be prevented by simultaneous injection of substrate for NO synthesis or exogenous source of NO. The reversal by NO of the ODQ-induced microvascular effects might be explained by the competitive interaction of NO and ODQ with the soluble form of guanylate cyclase (Garthwaite et al., 1995). Taken together, these results suggest that all the microcirculatory effects of L-NAME in awake hamster were due to inhibition of constitutive NO synthesis and the subsequent decrease in one or several cGMP-dependent regulatory pathway(s).

The effects of L-NAME on venule diameter could be temporally distinguished from its pro-inflammatory effects. Consistent with the rapid in vivo effects of L-NAME on haemodynamic parameters, venule diameter was immediately reduced whereas L-NAME-induced macromolecule extravasation did not begin for 60 min. In anaesthetized animals, superfusion of L-NAME or L-NMMA on exteriorized organs promotes leukocyte adhesion and migration by a reduction in NO synthesis and subsequent oxidative activation of mast cells (Kubes et al., 1991; Davenpeck et al., 1994; Kanwar et al., 1994; Suematsu et al., 1994; Kurose et al., 1995). The protective effects of constitutive NO synthesis could be mediated by a cGMP-dependent pathway since 8-bromo-cGMP reversed L-NAME-induced leukocyte adhesion in the rat mesentery (Davenpeck et al., 1994). Because leukocyte migration induced by superfusion of L-NAME or compound 48/80 is closely associated with a delayed increase in microvascular permeability in the rat mesentery (Kurose et al., 1995; Kubes and Gaboury, 1996), the late L-NAME-induced macromolecule extravasation that we observed in awake hamsters might result from such a mast cell-dependent mechanism. However, systemic application of L-NAME has numerous effects on haemodynamics which could alter the permeability of the microcirculation to macromolecules in a mast cell-independent manner, notably through a rise in hydrostatic pressure. Hydrostatic pressure results from a complex balance between precapillary and postcapillary vascular tone. Although L-NAME infusion rapidly increases mean arterial pressure, the hydrostatic pressure hypothesis would be highly speculative in this study since evaluation of the effects of L-NAME on arterioles was precluded by vasomotion in awake animals. Nevertheless, it has to be noted that the rapid effects of L-NAME on haemodynamics, as evidenced by the rapid reduction of venule diameter and rise in mean arterial pressure, should concomitantly increase hydrostatic pressure and associated macromolecule extravasation.

Infusion of L-arginine induced immediate macromolecule extravasation. D-arginine alone or simultaneous bolus injection of L-arginine with L-NAME failed to mimic the effects of L-arginine alone. The substrate of NO synthase, the NO donor, sodium nitroprusside and the cell-permeable analogue of cGMP, 8-bromo-cGMP, provoked rapid macromolecule extravasation. Inhibition of guanylate cyclase activity by ODQ prevented both L-arginine and sodium nitroprusside-induced macromolecule leakage. Thus, L-arginine effects on macromolecule extravasation would appear to be due to stimulation of constitutive NO synthesis and subsequent activation of one or several cGMP-dependent cascade(s). Consistent with our results, in isolated coronary venules, stimulation of constitutive endothelial NO synthase by flow produces a rapid rise in macromolecule permeability (Yuan et al., 1993). Furthermore, several studies have demonstrated that, in response to known inflammatory mediators such as histamine, there is a NO/cGMP-dependent formation of gaps between endothelial cells (Yuan et al., 1993; Mayhan, 1994). In our study, such a rapid stimulation of NO synthesis by L-arginine infusion and the subsequent rise in macromolecule permeability would not be associated with concomitant changes in haemodynamics. Indeed, neither mean arterial pressure nor venule diameter was rapidly altered after L-arginine injection. Furthermore, sodium nitroprusside and 8-bromo-cGMP induced rapid macromolecule leakage but did not modify venule diameter throughout the 180-min experiments. One possible explanation might be that, under our experimental conditions, plasma L-arginine,

sodium nitroprusside, and 8-bromo-cGMP stimulate the cGMP-dependent formation of gaps in the endothelial cell monolayer but fail to induce cGMP-mediated relaxation of vascular smooth muscle cells due to diffusion barriers. The mechanism of the L-arginine-induced late venule dilation remains obscure. Although D-arginine alone or simultaneous bolus injection of L-arginine with L-NAME failed to mimic the venule dilation caused by L-arginine alone, sodium nitroprusside and 8-bromo-cGMP did not affect venule dilation throughout the 180-min experiments. Thus, the involvement of the NO/cGMP-dependent cascade hypothesis in the L-arginine-induced venule dilation was not fully substantiated. In this regard, it has to be noted that L-arginine might have vascular effects independent of NO synthase activation (Durante et al., 1996; Wascher et al., 1997).

5. Conclusion

This intravital microscopy study provides the first evidence in the microcirculation of awake hamster for the opposing effects of basal nitric oxide synthesis on macromolecule extravasation. Both were due to temporally distinct cGMP-dependent mechanisms. Basal NO synthesis might protect against leukocyte adhesion and associated extravasation in non-inflammatory conditions. However, basal NO synthesis might control endothelial barrier function by the formation of gaps between endothelial cells.

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