British Journal of Pharmacology (1996) 118, 1371-1376

Bradykinin and changes in microvascular permeability in the hamster cheek pouch: role of nitric oxide

[1]M. Félétou, E. Bonnardel & E. Canet

Département de Pneumologie, Institut de Recherches Servier, 11 Rue des Moulineaux, 92150 Suresnes, France

1 The objective of this study in the hamster cheek pouch was to investigate the role of nitric oxide in bradykinin-induced microvascular leakage. The cheek pouch microcirculatory bed of the anaesthetized hamster was directly observed under microscope and vascular leakage was evidenced by dextran-fluorescein isothiocyanate (FITC-dextran) extravasation.

2 Bradykinin superfusion (but not [des-Arg^9]-bradykinin up to 3×10^{-6} M) induced an increase in microvascular permeability (log EC_{50}: -6.5 ± 0.4) which was exclusively located on the post-capillary venule. Plasma extravasation was blocked by intravenous pretreatment with Hoe 140, a bradykinin B_2 receptor antagonist (estimated log ID_{50}: -9.5 ± 0.2).

3 The effects of bradykinin (3×10^{-7} M) superfusion were partially but significantly inhibited by indomethacin (10^{-5} M, $P<0.05$) and abolished by pretreatment with L-nitro-arginine (L-NOARG; 10^{-5} M).

4 Acetylcholine (10^{-6} M, which releases endothelial nitric oxide (NO)) and sodium nitroprusside (10^{-6} M, a nitrovasodilator) superfusion did not induce any changes in permeability, *per se*. Cromakalim (10^{-5} M, a potassium channel opener) superfusion induced a moderate but significant plasma extravasation.

5 The effects of bradykinin, blocked by L-NOARG pretreatment, were restored by the co-perfusion of either sodium nitroprusside or cromakalim. Conversely vasoconstriction, produced by a stable analogue of thromboxane A_2 (U46619, 3×10^{-7} M), inhibited the increase in permeability produced by bradykinin.

6 The measurement of arteriolar diameter showed that bradykinin induced a vasodilatation which was blocked by L-NOARG. L-NOARG in itself was a powerful vasoconstrictor. Sodium nitroprusside and cromakalim, in the presence of L-NOARG, were able to restore the inhibited vasodilator response to bradykinin.

7 These results suggest: (1) bradykinin-induced microvascular leakage is mediated by bradykinin B_2 receptor activation; (2) the increase in permeability is due to two different independent phenomena, i.e. post-capillary venular endothelial gap formation and arteriolar vasodilatation which increases the post-capillary venular transmural pressure; (3) NO is only involved in the arteriolar dilatation component of the bradykinin-induced increase in microvascular permeability.

Keywords: Bradykinin B_2 receptor; microcirculation; permeability; nitric oxide; endothelium; Hoe 140; vasodilatation; plasma extravasation

Introduction

Bradykinin is a powerful endogenous endothelium-dependent vasodilator releasing prostaglandins, nitric oxide (NO) and other substances such as the unidentified endothelium-derived hyperpolarizing factor (Félétou *et al.*, 1994). This peptide is also a potent and ubiquitous pro-inflammatory peptide inducing the cardinal signs of inflammation: hyperalgesia, vasodilatation and vascular leakage (Bhoola *et al.*, 1992). The sequence of events that leads to an increase in vascular permeability is still unknown. In the hamster cheek pouch, a model which allows direct *in vivo* visualization of a microcirculatory bed with minimal surgical trauma, it has been demonstrated that local NO production is involved in the increase in permeability provoked by topical application of either bradykinin or histamine (Mayhan, 1992; 1994). However, in various *in vivo* inflammatory models (Filep *et al.*, 1993; Filep & Foldes-Filep, 1993; Kurose *et al.*, 1994; Laszlo *et al.*, 1995) or *in vitro* studies (Westendorp *et al.*, 1994; Draijer *et al.*, 1995) NO synthase inhibition leads to an increase in vascular permeability.

The purpose of this work was to determine, in the hamster cheek pouch, by which mechanisms bradykinin induces vascular leakage and to evaluate the contribution of endogenous NO production.

Methods

The method has been described in detail elsewhere (Svenjö *et al.*, 1978). Male Harlan golden hamsters (110-120 g; Gannat, France) were anaesthetized with sodium pentobarbitone (80 mg kg^{-1}; i.p.) and the body temperature was kept at 37°C (Ealing, Les Ullys, France). The trachea was cannulated, in order to facilitate the spontaneous breathing of the animal, and the femoral vein catheterized. The cheek pouch was exteriorized, dissected with care under microscope, fixed upon a Plexiglass perfusion chamber and superfused (6 ml min^{-1}, Ismatec, Zurich, Switzerland) with a modified Ringer's solution of the following composition (mM): NaCl 124, KCl 4.7, $CaCl_2$ 1.2, $MgSO_4$ 2, $NaHCO_3$ 25, HEPES 30 (pH 7.4; 36°C, bubbled with 5% CO_2-95% N_2 gas mixture). The cheek pouch could be observed under the microscope (modified Leitz Ergolux) either under visible light or under u.v. illumination. After a 30 min stabilization period, fluorescein isothiocyanate dextran (FITC dextran, 150 kD) was injected through the femoral vein (250 mg kg^{-1}, 0.5 ml 100 g^{-1}). Anaesthesia was maintained

[1] Author for correspondence.

with chlorax (chloralose: 25 g l^{-1} and borax 23 g l^{-1}, 0.1 ml; i.v.) when needed.

Then, 40 min later, bradykinin (3×10^{-7} M) was superfused for 5 min, with a precision pump (Precidor, Infors AG Basel, Switzerland) connected via a three-way valve to the superfusion line. Changes in permeability were evidenced by the appearance, under u.v. illumination of the preparation, of leakage sites situated on the postcapillary venules, and the accumulation of FITC dextran in the superfusion fluid, which was collected with a fraction collector (Eldex Lab. Inc., U.S.A.; 100 s intervals, 10 ml per fraction) and subsequently quantified with a spectrofluorimeter (495/520 nm, Perkins-Elmer). Total increase in FITC-extravasation (during the 5 min of bradykinin infusion and up to the return to basal level) or maximal increase in FITC-dextran extravasation (fraction containing the highest level of FITC) could be calculated. A strong correlation was found between the two ($r = 0.92$). Preparations with more than 10 spontaneous leakage sites (before the bradykinin superfusion) or with less than 80 or more than 200 leakage sites after the 5 min bradykinin infusion, were discarded (surface area of the microscope field: 0.2 cm^2). The preparation was washed out for 40 min; the number of leakage sites returned to zero and the extravasation of FITC dextran to its control value. We therefore maintained a 40 min wash-out period between each successive bradykinin exposure. Treatment with the bradykinin B_2 receptor antagonist Hoe 140 was performed i.v. in a cumulative manner. Each animal received the full range of Hoe 140 concentrations (0.05, 0.10, 0.50 and 1.50 nmol kg^{-1}). The first concentration of Hoe 140 was injected 10 min after the wash out of the first bradykinin superfusion; the second bradykinin superfusion being performed 30 min later. Successive Hoe 140 injections and bradykinin superfusions were performed following the same schedule.

Vascular diameter measurement

In a separate set of experiments, microvascular diameter measurement was performed by video image shearing and splitting (model 908, instrumentation for Physiology and Medicine, San Diego, CA, U.S.A.) as described by Intaglietta and Tompkins (1973). In each preparation studied, changes in internal diameter were measured in A2 (51.4 ± 3.2 μm), A3 (22.8 ± 2.2 μm) and A4 (11.1 ± 0.7 μm) type arterioles at precise sites which were located anatomically. A2, A3 and A4 type arterioles are successive branching arterioles, A4 being the smallest precapillary arterioles. Arteriolar diameter was measured before the infusion of drugs (control) and after the incubation period with L-nitro arginine (L-NOARG). During the superfusion of bradykinin, the diameter was measured 3 min after the beginning of the superfusion. Each vessel was its own control and changes in diameter are presented as %.

Substances used

Acetylcholine, bradykinin, [des-Arg^9]-bradykinin, L-nitro-arginine, L-arginine, D-arginine, indomethacin, sodium nitroprusside (Sigma, La verpillère, France). U 46619 (9,11-dideoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$; Cayman Chem. U.S.A.). Hoe 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin), cromakalim (IdRS, Suresnes, France). The drugs were dissolved in saline solution (Biosedra) with the exception of cromakalim and U46619, which were first dissolved in ethanol, and indomethacin which was prepared as a stock solution in an equimolar concentration of $NaCO_3$.

Statistical evaluation

Data are shown as means ± s.e.mean; n represents the number of animals studied. Statistical evaluation was performed by one or two way analysis of variance. When a significant interaction was observed ($P < 0.05$), a complementary analysis was undertaken (Newman-Keul's test) to identify differences between groups.

Results

Preliminary experiments

Bradykinin (3×10^{-8}, 10^{-7}, 3×10^{-7} and 10^{-6} M) was topically applied for 5 min in random order on the dissected hamster cheek pouch. Evaluation of the changes in microvascular permeability was performed either by counting the number of leaks or by measuring the FITC dextran extravasated in the superfusion fluid. Both methods gave similar results and showed that bradykinin induced a concentration-dependent increase in permeability (log EC_{50}: -6.5 ± 0.4, Figure 1). The EC_{50} bradykinin concentration (3×10^{-7} M) was chosen for the subsequent experiments. Five successive applications of bradykinin (3×10^{-7} M) induced reproducible results (Figure 2a). Both methods of measuring changes in permeability produced data which were qualitatively similar and significantly correlated ($r = 0.66$). Although both methods were used throughout this study, for the sake of clarity, data presented in the manuscript only involve the number of leakage sites.

Bradykinin receptor subtype

Two groups of hamsters, randomly assigned, were treated either with increasing doses of Hoe 140 (0.05, 0.10, 0.50 and 1.50 nmol kg^{-1}; i.v.) or with saline solution (0.1 ml kg^{-1}) administered 15 min before the topical bradykinin application. Hoe 140 treatment did not, *per se*, increase the hamster cheek pouch microvascular permeability (data not shown). In the saline treated group the five successive bradykinin (3×10^{-7} M) stimulations induced increases in permeability which were not significantly different. Hoe 140 treatment provoked a dose-dependent inhibition of the bradykinin-induced vascular leakage which was complete for the highest dose tested (estimated logID_{50}, assuming that Hoe 140 was neither metabolized nor eliminated during the experiment: -9.5 ± 0.2 as nmol kg^{-1}, $n = 5$; Figure 2a). In contrast, the increase in permeability produced by histamine (10^{-5} M) was unaffected by the highest dose of Hoe 140 (number of leaks: 58 ± 10 and 59 ± 1, respectively in control and treated animals, $n = 2$).

Topical application of [des-Arg^9]-bradykinin (3×10^{-7}, 10^{-6} and 3×10^{-6} M) did not produce any significant changes in microvascular permeability although bradykinin (3×10^{-7} M), applied before and after the [des-Arg^9]-bradykinin perfusions, induced vascular leakage (Figure 2b).

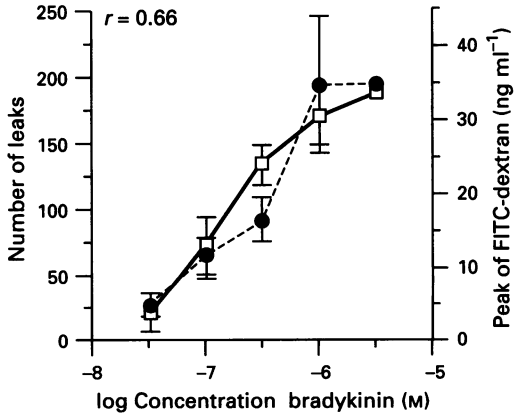

Figure 1 Bradykinin superfusion (5 min) and changes in microvascular permeability in the hamster cheek pouch. Data are shown as means ± s.e.mean. Bradykinin 3×10^{-8}, 10^{-7}, 3×10^{-7}, 10^{-6} and 3×10^{-6} M ($n = 7$, 8, 9, 9 and 1, respectively) were applied in random order. The number of vascular leakage sites was quantified after 5 min of bradykinin superfusion. The highest dose tested (3×10^{-6} M) appeared to be difficult to wash out and was tested only once. Peak in FITC-dextran extravasation (●–●) and number of vascular leaks (□–□) observed in the microscope field (20 mm^2) are shown.

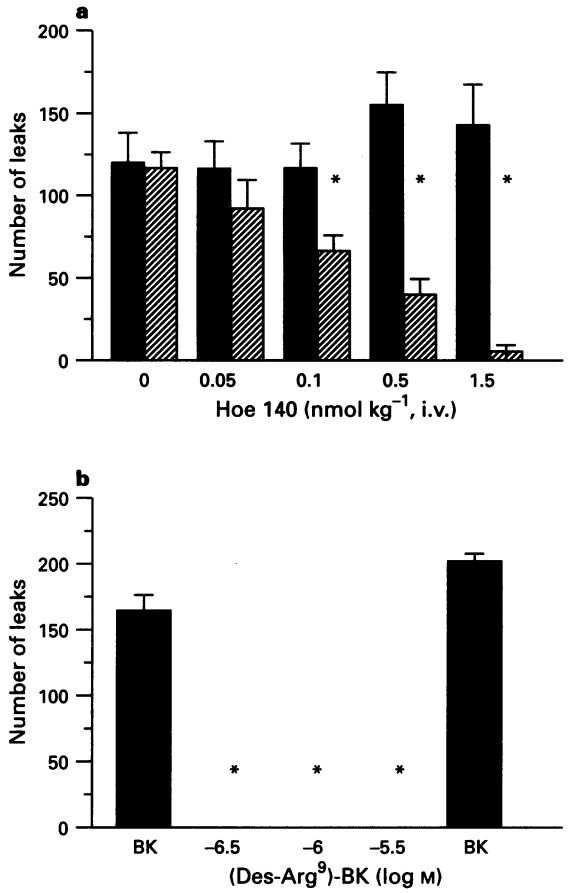

Figure 2 Bradykinin receptor subtype involved in changes in microvascular permeability in the hamster cheek pouch. (a) Effect of Hoe 140 treatment or saline (i.v.) on bradykinin superfusion-induced increase in microvascular permeability in the hamster cheek pouch. Data are shown as means ± s.e.mean and expressed as number of vascular leaks observed in the microscope field (20 mm^2), ($n=5$). *Indicates a statistically significant difference between saline- and Hoe 140-treated animals. In the saline-treated group (0.1 ml kg^{-1}, i.v., (solid columns) bradykinin (3×10^{-7} M) was infused for 5 min and induced a reproducible response. Hoe 140 (0.05, 0.10, 0.50 and 1.50 nmol kg^{-1}, i.v.), injected in a cumulative manner every 45 min, induced a dose-dependent inhibition of the changes in microvascular permeability produced by the bradykinin superfusion (hatched columns). (b) Effect of [des-Arg^9]-bradykinin on microvascular leakage in the hamster cheek pouch. Data are shown as means and s.e.mean and expressed as the number of vascular leaks observed in the microscope field (20 mm^2), ($n=3$). *Indicates a statistically significant difference between [des-Arg^9]-bradykinin and bradykinin. [Des-Arg^9]-bradykinin, up to a concentration of 3×10^{-6} M, did not induce a significant increase in microvascular leakage. Bradykinin (3×10^{-7} M) superfused at the beginning and the end of the experimental procedure is also shown as a positive control.

Cyclo-oxygenase and NO synthase inhibition

Topical superfusion of indomethacin (10^{-5} M) induced a modest but significant inhibition of the changes in permeability provoked by bradykinin (number of leaks: 127 ± 10 and 98 ± 8, respectively in control and indomethacin-treated cheek pouches, $P<0.05$, $n=4$). L-Nitro-arginine (L-NOARG, 10^{-5} M) topical superfusion completely abolished the increase in permeability provoked by bradykinin. L-NOARG (10^{-5} M) plus D-arginine (3×10^{-4} M) superfusion of the hamster cheek pouch did not produce any changes in the inhibition compared to that provoked by L-NOARG alone. However, superfusion of the pouches with both L-NOARG (10^{-5} M) and L-arginine (3×10^{-4} M) restored the increase in permeability provoked by bradykinin (Figure 3). Indomethacin, L-NOARG, L-arginine and D-arginine did not provoke vascular leakage.

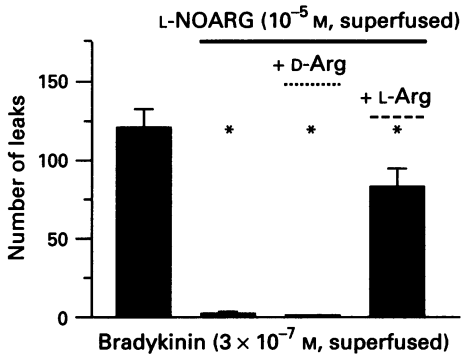

Figure 3 Effect of NO synthase inhibition on bradykinin-induced leakage. Data are shown as means and s.e.mean and expressed as the number of vascular leaks observed in the microscope field (20 mm^2), ($n=9$). *Indicates a statistically significant difference between control and treated preparations. Bradykinin (3×10^{-7} M, superfusion) induced an increase in microvascular leakage which was inhibited by L-nitro-arginine (L-NOARG: 10^{-5} M, superfusion started 30 min before the second bradykinin administration and maintained throughout the rest of the experiment). The addition of D-arginine (3×10^{-4} M, started 30 min before the third bradykinin administration) had no effect on the response to L-NOARG. However, L-arginine (3×10^{-4} M, started 30 min before the fourth bradykinin administration) restored the increase in microvascular permeability evoked by bradykinin.

Endothelium-dependent and independent vasodilators and changes in permeability

The intrinsic effects of the topical superfusion of three vasodilators, acetylcholine (10^{-6} M, $n=4$) sodium nitroprusside (10^{-6} M, $n=7$) and cromakalim (10^{-5} M, $n=4$), were studied (Figure 4a). Acetylcholine and sodium nitroprusside did not induce any significant changes in permeability (number of leaks 0 ± 0 and 1 ± 0.5, respectively). Cromakalim superfusion induced a moderate but significant plasma extravasation (number of leaks: 39 ± 15). Ethanol, the solvent, did not provoke any significant increase in permeability.

In the presence of L-NOARG (10^{-5} M), superfusion of either sodium nitroprusside (10^{-6} M) or cromakalim (10^{-5} M) provoked restoration of the vascular leakage produced by bradykinin (3×10^{-7} M), which was previously inhibited by the NO synthase inhibitor (Figure 4b).

Vasoconstriction and changes in permeability

Superfusion of the thromboxane A_2 analogue, U 46619 (3×10^{-7} M), induced either no changes or, in one experiment, a moderate increase in vascular leakage (number of leaks: 16 ± 16, $n=4$). In the presence of U 46619, the increase in permeability induced by bradykinin (3×10^{-7} M) was significantly reduced when compared to control conditions (145 ± 23 and 50 ± 29, respectively, in control and in the presence of U 46619; $P<0.05$).

Vascular diameter measurement

Bradykinin (3×10^{-7} M) produced a significant vasodilatation especially in A4 arterioles. L-NOARG treatment (10^{-5} M) provoked a significant and comparable vasoconstriction in arterioles. In the presence of L-NOARG (10^{-5} M), the effect of bradykinin was significantly inhibited in A2 and A4 arterioles (Figure 5).

In the presence of L-NOARG, superfusion of either sodium nitroprusside (10^{-6} M) or cromakalim (10^{-5} M) provoked the restoration of the vasodilatation produced by bradykinin (3×10^{-7} M), which was previously inhibited by the NO synthase inhibitor (Figure 6a and b).

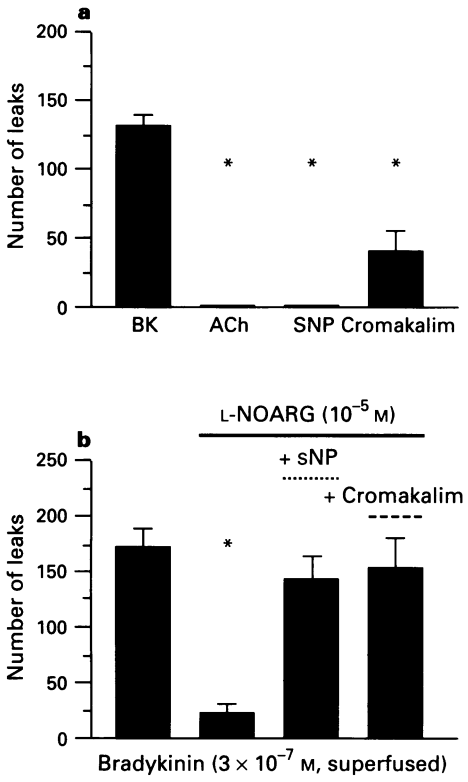

Figure 4 Effect of vasodilators on changes in microvascular leakage in the hamster cheek pouch. (a) Direct effect of different vasodilators. Data are shown as means and s.e.mean and are expressed as the number of vascular leaks observed in the microscope field (20 mm^2). Bradykinin (BK: 3×10^{-7} M, first superfusion, $n=8$), acetylcholine (ACh: 10^{-6} M, $n=4$), sodium nitroprusside (SNP: 10^{-6} M, $n=7$), cromakalim (10^{-5} M, $n=5$) were superfused. *Indicates a statistically significant difference between the effects of the vasodilators and bradykinin. (b) Effect of vasodilators on changes in microvascular leakage induced by bradykinin in the presence of NO-synthase inhibition. Data are shown as means and s.e.mean and expressed as the number of vascular leaks observed in the microscope field (20 mm^2). Bradykinin (BK: 3×10^{-7} M, first superfusion, $n=17$); bradykinin in the presence of L-nitro-arginine (L-NOARG: 10^{-5} M, $n=12$); bradykinin in the presence of L-NOARG and sodium nitroprusside (SNP: 10^{-6} M, $n=5$); bradykinin in presence of L-NOARG and cromakalim (10^{-5} M, $n=5$). *Indicates a statistically significant difference in L-NOARG-treated preparations in the absence and presence of vasodilators.

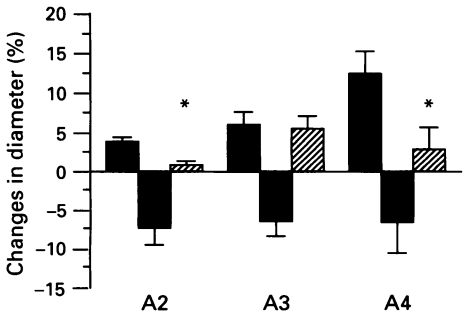

Figure 5 Effect of bradykinin and L-NOARG on arteriolar diameter of the hamster cheek pouch. Data are shown as means and s.e.mean and expressed as % of variation in diameter. For each arteriolar type (A2, A3, and A4, respective initial diameter: 51.9 ± 4.3, 22.0 ± 2.9, 11.5 ± 0.9 μm), the first column represents the effect of bradykinin (3×10^{-7} M; $n=15$; solid columns), the second the effect of L-NOARG (10^{-5} M, $n=9$; stippled columns) and the third the effect of bradykinin in the presence of L-NOARG ($n=9$; hatched columns).

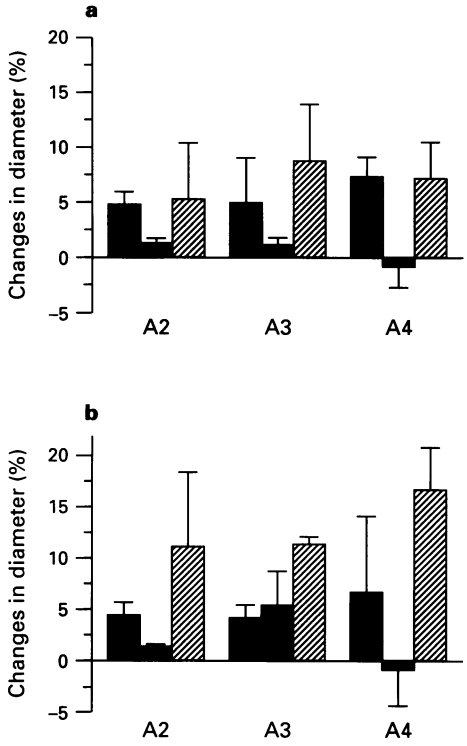

Figure 6 Effect of bradykinin and L-NOARG on changes in the arteriolar diameter of the hamster cheek pouch in the presence of vasodilators. (a) Effect of bradykinin (3×10^{-7} M) in the presence of L-NOARG (10^{-5} M) and sodium nitroprusside (SNP: 10^{-6} M). Initial diameter for A2, A3 and A4 arterioles: 46.8 ± 2.7, 20.0 ± 0.8 and 12.4 ± 1.8 μm, respectively. (b) Effect of bradykinin in the presence of L-NOARG and cromakalim (10^{-5} M). Initial diameter for A2, A3, and A4 arterioles: 43.7 ± 1.3, 16.3 ± 1.2 and 11.2 ± 2.3 μm, respectively. Data are shown as means and s.e.mean and are expressed as % of variation in diameter, ($n=3$). For each arteriolar type (A2, A3 and A4), the first column represents the effect of bradykinin (solid columns), the second the effect of bradykinin in the presence of L-NOARG (stippled columns) and the third the effect of bradykinin in the presence of L-NOARG and sodium nitroprusside 10^{-6} M (a, hatched columns) or in the presence of L-NOARG and cromakalim 10^{-5} M (b, hatched columns).

Discussion

The hamster cheek pouch has been extensively used as a model to study microcirculation and changes in microvascular permeability (Duling, 1973; Svenjö *et al.*, 1978). Preliminary experiments demonstrated that the hamster cheek pouch preparation was stable over time and that bradykinin could be successfully reapplied five consecutive times, allowing each animal to act as its own control (Gawlowski *et al.*, 1982). The dose-dependent increase in permeability provoked by bradykinin observed in this study and the results obtained with the two methods of evaluating the changes in permeability (e.g. number of leaks and spectrofluorimetric determination of extravasated FITC dextran, and the correlation between the two) were similar to previous findings (Gawlowski *et al.*, 1982; Svenjö, 1990).

Hoe 140, the potent selective, long-acting B_2 bradykinin receptor antagonist, fully inhibited the effects of bradykinin as previously published (Hock *et al.*, 1991; Wirth *et al.*, 1991; Félétou *et al.*, 1994; 1995a,b and c). Furthermore, [des-Arg^9]-bradykinin, a specific B_1 receptor agonist did not have any effect in the hamster cheek pouch (Regoli & Barabe, 1980). So in our experimental conditions, the bradykinin-induced increase in microvascular permeability can be fully attributed to bradykinin B_2 receptor stimulation, confirming earlier results (Murray *et al.*, 1991; Félétou *et al.*, 1995c).

Topical administration of indomethacin induced a small

inhibition of the increase in microvascular permeability evoked by bradykinin, indicating that the involvement of the cyclo-oxygenase-derived metabolites of arachidonic acid is minimal (Gao *et al.*, 1993; Mayhan & Rubinstein, 1993). However, we observed that local treatment of the hamster cheek pouch with a nitric oxide synthase inhibitor completely prevented the effects of bradykinin, suggesting a major contributory role for the L-arginine-NO-dependent pathway as previously demonstrated by Mayhan *et al.* (1992, 1994). The stereo-specific restoration of bradykinin-induced vascular leakage, in the presence of L-NOARG, by L-arginine confirms the involvement of NO synthase. Bradykinin is one of the most potent mediators inducing vascular relaxation by the release of endothelial NO (Cherry *et al.*, 1982; Félétou *et al.*, 1995a,b).

The mechanism by which bradykinin and NO modulate protein and fluid leakage has not been definitively established. The involvement of blood flow modulation in the increase in microvascular permeability is a matter of controversy. In the hamster cheek pouch bradykinin induced a vasodilatation, especially in precapillary A4 arterioles, which was blocked by L-NOARG. Acetylcholine, the archetype of endothelium-dependent mediators (Furchgott & Zawadzki, 1980) produced NO synthase inhibitor-sensitive vasodilatation in the hamster cheek pouch microcirculatory bed (Mayhan, 1993, Félétou *et al.*, unpublished observations). However, in contrast to bradykinin, the muscarinic agonist did not induce any changes in microvascular permeability. Similarly, two other vasodilators, cromakalim (potassium channel opener) and sodium nitroprusside (nitrovasodilator) produced minor or no plasma extravasation, although both compounds induced vasodilatation in the hamster cheek pouch (Mayhan, 1993; Jackson, 1993; Hall & Brain, 1994). These data demonstrate that, in the hamster cheek pouch, vasodilatation is not sufficient to induce vascular leakage and also that, in this preparation, NO, either endogenously released (acetylcholine) or exogenously delivered (sodium nitroprusside), does not, *per se*, produce an increase in microvascular permeability. In L-NOARG treated preparations, the inhibition of bradykinin-induced plasma extravasation could be logically reversed by the reintroduction of NO (sodium nitroprusside). However, the L-NOARG inhibitory effect was also reversed by a NO-independent vasodilator, cromakalim. These results suggest that, in order to increase microvascular permeability, bradykinin acts at, at least, two different sites. It has been demonstrated that, at the site of vascular leakage (e.g. post-capillary venules), bradykinin, along with other inflammatory mediators, induces the formation of endothelial gaps by a mechanism which is not yet properly defined (Majno & Palade, 1961; Hulstrom & Svenjö, 1979; Hammersen & Hammersen, 1987; McDonald, 1994; Hirata *et al.*, 1995). Nitric oxide is probably not involved in this effect of bradykinin since in the absence of NO production (presence of L-NOARG), but in the presence of a vasodilator which does not release NO, such as cromakalim, bradykinin-induced vascular leakage was restored.

The second mechanism necessary for bradykinin-induced increase in permeability, is probably vasodilatation and a subsequent increase in local blood flow. Indeed L-NOARG, which induces vasoconstriction and inhibits the vasodilatation produced by bradykinin, blocked the changes in permeability provoked by this inflammatory mediator. U 46619, a potent vasoconstrictor in the hamster cheek pouch microcirculatory bed (Mayhan & Rubinstein, 1995), was also able to inhibit the changes in permeability provoked by bradykinin. In L-NOARG-treated preparations, sodium nitroprusside and cromakalim restored both the vasodilator and vascular leakage responses to bradykinin. Indeed arteriolar vasodilatation increases the microvascular pressure in A4 arterioles, capillaries and post-capillary venules (Davis & Gore, 1985). In the hamster cheek pouch, adenosine provokes arteriolar dilatation, increases in microvascular pressure (Davis & Gore, 1985) and microvascular permeability (Mayhan, 1992). These events are unaffected by NO-synthase inhibition (Mayhan, 1992), adenosine being in most vascular beds an endothelium-independent vasodilator. In addition, Medeiros *et al.* (1995) have also suggested that the inhibtory effect of NO synthase inhibitors on carrageenin-induced plasma extravasation (a phenomenon inhibited by bradykinin B_2 receptor antagonists; Félétou *et al.*, 1995a) reflected a local decrease in blood flow rather than a direct effect on vascular permeability.

However, it has been proposed that, in the hamster cheek pouch, NO could modulate microvascular permeability by a mechanism which is independent of changes in local blood flow. Some agents, such as platelet activating factor (PAF), induce arteriolar vasoconstriction and NO synthase-dependent plasma extravasation (Dillon *et al.*, 1988; Dillon & Duran, 1988; Ramirez *et al.*, 1995). However, the time course of the two events could be dissociated, vasoconstriction (attributed to thromboxane A_2 activity) being transient and vascular diameters reverting to near control values at the peak of plasma extravasation (Dillon *et al.*, 1988; Dillon & Duran, 1988). Furthermore, the concentration-response curve for PAF-induced plasma extravasation (attributed to leukotriene production) is bell-shaped while the concentration-response curve to PAF-induced vasoconstriction is monophasic; e.g. the elevated concentration of PAF (10^{-5} M) which induced the highest degree of vasoconstriction could not induce vascular leakage (Dillon *et al.*, 1988; Dillon & Duran, 1988). Finally, the arteriolar vasoconstriction to PAF could be pharmacologically inhibited while the postcapillary venular permeability response was preserved (Tomeo & Duran, 1991). Nevertheless, in this case NO production cannot be directly linked to a vasodilator effect, possibly because of the pleiotropic action of PAF.

In conclusion, our results suggest that in the hamster cheek pouch: (1) bradykinin-induced microvascular leakage is mediated by bradykinin B_2 receptor activation; (2) the increase in permeability is due to two different independent phenomena, i.e. post-capillary venular endothelial gap formation and arteriolar vasodilatation which increases the post-capillary venular transmural pressure; (3) NO is only involved in the arteriolar dilatation component of the bradykinin-induced increase in microvascular permeability.

We thank Dr E. Bouskela for her help in the development of the hamster cheek pouch model in our institution and M.O. Vallez for her skilful assistance. We thank Dr E. Svenjö for fruitful comments and discussion.

References

BHOOLA, K.D., FIGUEROA, C.D. & WORTHY, K. (1992). Bioregulation of kinins: kallikreins, kininnogens, and kininases. *Pharmacol. Rev.*, **44**, 1–80.

CHERRY, P.D., FURCHGOTT, R.F., ZAWADZKI, J.V. & JOTHIANANDAN, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad Sci. U.S.A.*, **79**, 2106–2110.

DAVIS, M.J. & GORE, R.W. (1985). Pressure distribution in the microvascular network of the hamster cheek pouch. In *Microvascular Networks: Experimental and Theoretical Studies*, ed. Popel, A.S. & Jonson, B., pp. 142–154, Basel: Karger.

DILLON, P.K. & DURAN, W.N. (1988). Effect of platelet-activating factor on microvascular permselectivity: dose-response relations and pathways of action in the hamster cheek pouch microcirculation. *Circ. Res.*, **62,** 732–740.

DILLON, P.K., RITTER, A.B. & DURAN, W.N. (1988). Vasoconstrictor effects of platelet-activating factor in the hamster cheek pouch microcirculation: Dose related relations and pathways of action. *Circ. Res.*, **62,** 722–731.

DRAIJER, R., ATSMA, D.E., VANDERLAARSE, A. & VANHINSBERGH, V.W.M. (1995). c-GMP and nitric oxide modulate thrombin-induced endothelial permeability–Regulation via different pathways in human aortic and umbilical vein endothelial cells. *Circ. Res.*, **76,** 199–208.

DULING, B.R. (1973). The preparation and use of the hamster cheek pouch for studies of the microcirculation. *Microvasc. Res.*, **5,** 423–429.

FÉLÉTOU, M., CANET, E. & VANHOUTTE, P.M. (1993). NO, Endothélium et Régulation vasculaire. *Réan. Urg.*, **2,** 609–624.

FÉLÉTOU, M., GERMAIN, M., THURIEAU, C., FAUCHÈRE, J.L. & CANET, E. (1994). Agonistic and antagonistic properties of the bradykinin B_2 receptor antagonist, HOE 140 in isolated blood vessels from different species. *Br. J. Pharmacol.*, **112,** 683–689.

FÉLÉTOU, M., LONCHAMPT, M., ROBINEAU, P., JAMONNEAU, I., THURIEAU, C., FAUCHÈRE, J-L., VILLA, P., GHEZZI, P., PROST, J-F. & CANET, E. (1995c). Effect of the bradykinin B2 receptor antagonist S 16118 (p-Guanidobenzoyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin) in different *in vivo* models of inflammation. *J. Pharmacol. Exp. Ther.*, **273,** 1078–1084.

FÉLÉTOU, M., MARTIN, C.A.E., MOLIMARD, M., NALINE, E., GERMAIN, M., THURIEAU, C., FAUCHÈRE, J-L., CANET, E. & ADVENIER, C. (1995a). In vitro effects of HOE 140 in human bronchial and vascular tissue. *Eur. J. Pharmacol.*, **274,** 57–64.

FÉLÉTOU, M., ROBINEAU, P., LONCHAMPT, M., BONNARDEL, E., THURIEAU, C., FAUCHÈRE, J-L., WIDDOWSON, P., MAHIEU, J-P., SERKIZ, B., VOLLAND, J-P., MARTIN, C., NALINE, E., ADVENIER, C., PROST, J-F. & CANET, E. (1995b). S 16118 (p-Guanidobenzoyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin) is a potent and long acting bradykinin B_2 receptor antagonist, *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, **273,** 1071–1077.

FILEP, J.G. & FOLDES-FILEP, E. (1993). Modulation by nitric oxide of platelet activating factor-induced albumin extravasation in the conscious rat. *Br. J. Pharmacol.*, **110,** 1347–1352.

FILEP, J.G., FOLDES-FILEP, E., ROUSSEAU, A., SIROIS, P. & FOURNIER, A. (1993). Vascular responses to endothelin-1 following inhibition of nitric oxide synthesis in the conscious rat. *Br. J. Pharmacol.*, **110,** 1213–12121.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373–376.

GAO, X.P., MAYHAN, W.G., CONLON, J.M., RENNARD, S.I. & RUBINSTEIN, I. (1993). Mechanisms of T-kinin-induced increases in macromolecule extravasation in vivo. *J. Appl. Physiol.*, **74,** 2896–2903.

GAWLOWSKI, D.M., RITTER, A.B. & DURAN, W.N. (1982). Reproductibility of microvascular permeability responses to successive topical applications of bradykinin in the hamster cheek pouch. *Microvasc. Res.*, **24,** 354–363.

HALL, J.M. & BRAIN, S.D. (1994). Inhibition by SR140333 of NK1 tachykinin receptor-evoked nitric oxide-dependent vasodilation in the hamster cheek pouch microvasculature in vivo. *Br. J. Pharmacol.*, **113,** 522–526.

HAMMERSEN, F. & HAMMERSEN, E. (1987). The ultrastructure of endothelial gap-formation and leukocyte emigration. *Prog. Appl. Microcirc.*, **12,** 1–34.

HIRATA, A., BALUK, P., FUJIWARA, T. & MCDONALD, D.M. (1995). Location of silver staining at endothelial gaps in inflamed venules examined by scanning electron microscopy. *Am. J. Physiol.*, **13,** L403–L418.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new, potent and long-acting bradykinin antagonist: in vitro studies. *Br. J. Pharmacol.*, **102,** 769–773.

HULSTRÖM, D. & SVENJÖ, E. (1979). Intravital and electron microscopic study of bradykinin-induced vascular permeability changes using FITC-dextran as a tracer. *J. Pathol.*, **129,** 125–133.

INTAGLIETTA, M. & TOMPKINS, W.R. (1973). Microvascular measurements by video image shearing and splitting. *Microvasc. Res.*, **5,** 309–312.

JACKSON, W.F. (1993). Arteriolar tone is determined by activity of ATP-sensitive potassium channels. *Am. J. Physiol.*, **265,** H1797–H1803.

KUROSE, I., WOLF, R., GRISHAM, M.B. & GRANGER, D.N. (1994). Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. *Circ. Res.*, **74,** 376–382.

LASZLO, F., WHITTLE, B.J.R. & MONCADA, S. (1995). Attenuation by nitrosothiol NO donnors of acute intestinal microvascular dysfunction in the rat. *Br. J. Pharmacol.*, **115,** 498–502.

MAJNO, G. & PALADE, G.E. (1961). Studies on inflammation. The effects of histamine and serotonin on vascular permeability: an electron microscopy study. *J. Biophys. Biochem. Cytol.*, **11,** 571–605.

MAYHAN, W.G. (1992). Role of nitric oxide in modulating permeability of hamster cheek pouch in response to adenosine-5-diphosphate and bradykinin. *Inflammation*, **16,** 295–304.

MAYHAN, W.G. (1993). In vivo reactivity of resistance arterioles to activation of ATP-sensitive K+ channels. *Eur. J. Pharmacol.*, **242,** 109–112.

MAYHAN, W.G. (1994). Nitric oxide accounts for histamine-induced increases in macromolecular extravasation. *Am. J. Physiol.*, **266,** H2369–H2373.

MAYHAN, W.G. & RUBINSTEIN, I. (1993). Cigarette smoke extract potentiates bradykinin–induced in microvascular permeability. *J. Appl. Physiol.*, **75,** 27–32.

MAYHAN, W.G. & RUBINSTEIN, I. (1995). Response of hamster cheek pouch microcirculation to endothelin and endothelin receptor antagonists. *Int. J. Microcirc.*, **15,** 48–52.

MCDONALD, D. (1994). Endothelial gaps and permeability of venules in rat tracheas exposed to inflammatory stimuli. *Am. J. Physiol.*, **266,** L61–L83.

MEIDEIROS, M.V., BINHARA, I.M., MORENO, H., ZATZ, R., DE NUCCI, G. & ANTUNES, E. (1995). Effect of chronic nitric oxide synthesis inhibition on the inflammatory responses induced by carrageenin in rats. *Eur. J. Pharmacol.*, **285,** 109–114.

MURRAY, M.A., HEISTAD, D.D. & MAYHAN, W.G. (1991). Role of protein kinase C in bradykinin-induced increases in microvascular permeability. *Circ. Res.*, **68,** 1340–1348.

RAMIREZ, M.M., QUARDT, S.M., KIM, D., OSHIRO, H., MINNICOZZI, M. & DURAN, W.N. (1995). Platelet activating factor modulates microvascular permeability through nitric oxide synthesis. *Microvasc. Res.*, **50,** 223–234.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32,** 1–46.

SVENJÖ, E. (1990). The hamster cheek pouch as a model in microcirculation research. *Eur. Respir. J.*, **3,** 595s–601s.

SVENJÖ, E., ARFORS, K.E., ARTURSON, G. & RUTILI, G. (1978). The hamster cheek pouch as a model for studies of macromolecular permeability of the microvasculature. *Ups. J. Med. Sci.*, **83,** 71–79.

TOMEO, A.C. & DURAN, W.N. (1991). Resistance and exchange microvessels are modulated by different PAF receptors. *Am. J. Physiol.*, **261,** H1648–H1652.

WESTENDORP, R.G.J., DRAIJER, R., MEINDERS, A.E. & VANHINSBERGH, V.W.M. (1994). Cyclic-GMP-mediated decrease in permeability of human umbilical and pulmonary artery endothelial cell monolayers. *J. Vasc. Res.*, **31,** 42–51.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANNN, H.G., ANAGNOSTOPOULOS, H., HENKE, S., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin antagonist: *in vivo* studies. *Br. J. Pharmacol.*, **102,** 774–777.

(Received November 22, 1995
Revised March 20, 1996
Accepted March 28, 1996)