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Characterization of receptors for kinins and neurokinins in the arterial and venous mesenteric vasculatures of the guinea-pig

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1 In the present work, we have studied the microvascular reactivity of the arterial and venous mesenteric beds of the guinea-pig to bradykinin, neurokinins and other agents.

2 The vasoactive properties of three selective agonists for neurokinin receptors, namely [Sar^9,Met$(O_2)^{11}$]SP (NK_1), [β-Ala^8]NKA(4–10) (NK_2) and [MePhe^7]NKB (NK_3), were evaluated on precontracted arterial and venous mesenteric vasculatures of the guinea-pig. The NK_1-selective agonist, [Sar^9,Met$(O_2)^{11}$]SP (1 to 1000 pmol), induced an endothelium-dependent and N^{ω}-nitro-L-arginine methyl ester (L-NAME)-sensitive relaxation of the arterial vasculature precontracted with methoxamine, whereas the NK_2 and NK_3-selective agonists were virtually inactive at high doses (1000 pmol).

3 The three selective neurokinin receptor agonists were inactive in the non-precontracted arterial and venous mesenteric vasculatures as well as in the precontracted venous mesenteric vasculature.

4 Bradykinin (0.1 to 100 pmol) induced a marked dose- and endothelium-dependent vasodilatation of the precontracted arterial and venous vasculatures. ED_{50} values were 5.5 pmol on the arterial side and 1.9 pmol on the venous side. In contrast, desArg^9-bradykinin was inactive at doses up to 1000 pmol. Furthermore, on the arterial and venous sides, a higher dose of bradykinin (1000 pmol), induced a biphasic effect, a transient constriction followed by a marked and sustained vasodilatation. The vasodilator effects of bradykinin were abolished by Hoe 140 (0.1 μM) and CHAPS, markedly reduced by L-NAME and were unaffected by [Leu^8]desArg^9-bradykinin (0.1 μM) on both sides of the mesenteric vasculature. Hoe 140 also abolished the arterial vasoconstrictions induced by high doses of bradykinin.

5 Noradrenaline, angiotensin II and endothelin-1 produced contractions on both sides of the mesenteric circulation, while acetylcholine (arterial side) and sodium nitroprusside (arterial and venous sides) caused vasodilatation.

6 Our study supports the view that NK_1 receptors responsible for vasodilatation are present solely in the endothelium of the arterial mesenteric vasculature of the guinea-pig. On the other hand, bradykinin (0.1 to 100 pmol) exerts predominantly vasodilator effects on both sides of the mesenteric vasculature via selective activation of B_2 receptors located on the endothelium. The same receptor type located on the smooth muscle appears to be responsible for the arterial and venous constriction with high doses of bradykinin.

Keywords: Kinin and neurokinin receptors; guinea-pig mesenteric bed; vasodilatation; vasoconstriction

Introduction

Using the simultaneously perfused mesenteric arterial and venous beds (Warner, 1990), we have characterized receptors for the endothelins (ET_A and ET_B; Warner *et al.*, 1989; D'Orléans-Juste *et al.*, 1993), calcitonin gene-related peptide (Claing *et al.*, 1992), neurokinins (D'Orléans-Juste *et al.*, 1991) and platelet-activating factor (PAF) (Claing *et al.*, 1994) in the rat. This model has the advantage that the responses of the arterial and venous vessels may be compared at the same time, helping for instance, to demonstrate how pro-inflammatory agents, such as PAF, cause concurrently arterial vasodilatation and venoconstriction and so regulate the hydrostatic pressure within the capillary bed (Claing *et al.*, 1994). In addition, the double-perfused model described by Warner (1990) allows the monitoring of venodilatations by agents such as kinins, in vasculatures with a functional and intact endothelium.

On the other hand, the arterial and venous reactivity to vasoactive agents has yet to be investigated in the guinea-pig mesentery. To our knowledge, the only study comparing endothelium-dependent arterial and venous reactivity in the guinea-pig has been performed with acetylcholine in rings of femoral origin, as reported by Suh *et al.* (1992). Hence, it is of interest to develop the guinea-pig counterpart of the simultaneously perfused rat mesenteric vascular bed. In such models, one can study the effects of vasoactive drugs, as well as the response to sympathetic and non-adrenergic non-cholinergic perivascular nerve stimulation (Warner *et al.*, 1990; Claing *et al.*, 1992) in endothelium-intact pre and post-capillary vasculatures.

Here, we have compared the venous and arterial effects of kinins and neurokinins in the mesenteric vasculature of the guinea-pig since quantitative or qualitative differences in the responses to various vasoactive agents compared to the rat may indicate, among other considerations, possible species differences in their pro-inflammatory potential.

Methods

The guinea-pig mesenteric vascular bed perfused simultaneously through the arterial and venous sides

The guinea-pig mesentery was prepared as described for the rat (McGregor, 1965; Warner, 1990). Briefly, Dunkin Hartley guinea-pigs of either sex (250–350 g) were killed by stunning and exsanguination. The abdomen was opened and the ileocolic and colic branches of the superior mesenteric artery tied.

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The portal-mesenteric vein was freed of connective and adipose tissues and this vessel and the superior mesenteric artery cannulated (Portex size tube 3FG). The mesentery was then perfused (2 ml min^{-1} for 5 min) via the mesenteric artery with warmed and gassed (37.5°C, 95% O_2 and 5% CO_2) Krebs solution containing heparin (100 units ml^{-1}). Following this initial perfusion period, the mesentery was separated from the intestine by cutting close to the intestinal border and the venous and arterial vasculatures perfused independently at flow rates of 2 ml min^{-1} with Krebs solution containing indomethacin (5 μM). In all other experiments, the perfusion flow rates were increased to 6 ml min^{-1} and the responses of the vasculature to bradykinin (BK), noradrenaline (NA), angiogensin II (AII) and endothelin-1 (ET-1), administered intraluminally through lateral injection ports, were measured with pressure transducers (Statham, Model P-23A) and recorded on a Grass physiograph (Model 7-D). In preliminary studies, vascular reactivity to endothelin-1 was assessed in the absence and presence of indomethacin, following which all other experiments were performed in the presence of the non-steroidal anti-inflammatory agent.

Endothelium-dependent vasodilatation

In order to evaluate the endothelium-dependent relaxant effects of neurokinin analogues, bradykinin, and acetylcholine (ACh), the perfusion pressure on both sides of the mesenteric circulation was increased by infusing on the venous side the thromboxomimetic, U46619 (9,11-dideoxy-9α,11α-epoxymethano prostaglandin $F_{2\alpha}$) (4.2 μM), and on the arterial side the sympathomimetic, methoxamine (200 μM). The perfusion flow rate was increased to 6 ml min^{-1} on both sides of the mesenteric circulation to reduce spontaneous activity. This latter perfusion flow was also chosen following preliminary experiments where the vascular reactivity to methoxamine (arterial) and U46619 (venous) was monitored at rates of 2, 6 and 9 ml min^{-1}. When steady increase of perfusion had been obtained on both sides of the circulation, the various peptides were administered as bolus injections. The apparent affinities of [Sar^9,$Met(O_2)^{11}$]SP and bradykinin were obtained by monitoring the vasoactive responses of the mesenteric vasculature to increasing doses of these agonists, in absence or presence of increasing concentrations of their respective antagonists (CP-99,994 for [Sar^9,$Met(O_2)^{11}$]SP and Hoe 140 for BK). ED_{50} (for the agonists) and IC_{50} (for the antagonists) were calculated from a linear regression analysis (D'Orléans-Juste *et al.*, 1993; Claing *et al.*, 1994). [Sar^9,$Met(O_2)^{11}$]SP, bradykinin and sodium nitroprusside (NaNP) were also tested in absence or presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 200 μM, 30 min) or CHAPS (20 mM, 30 s).

Venous and arterial contractions of the mesenteric vasculature in response to various peptides

Following an equilibration period of 60 min, the perfusion flow rate was adjusted to 6 ml min^{-1} and the pressor effects of three selective neurokinin analogues, bradykinin, angiotensin II, noradrenaline and endothelin-1 were measured on both sides of the mesenteric vasculature. Agonists were administered by bolus injections in volumes of 1–10 μl. In some experiments, L-NAME (200 μM, 30 min), Hoe 140 (D-Arg-[Hyp^3, Thi^5,D-Tic^7,Oic^8]BK) (0.001 to 0.1 μM, 15 min), [Leu^8]des-Arg^9BK (0.1 μM, 15 min) or CP-99,994 ((+)-(2S,3S)-3-(2-methoxy-benzylamino)-2-phenylpiperidine) (0.0005 to 0.5 μM, 15 min) were infused prior to the administration of the various agonists. Individual drugs were administered consecutively at time intervals of 5 to 50 min to avoid tachyphylaxis.

Drugs

All peptides except endothelin-1 were synthetised in our laboratory by D. Jukic by the solid-phase method (Drapeau & Regoli, 1988). Noradrenaline ((-)arterenol bitartrate), indomethacin, atropine, methysergide, diphenhydramine, N^{ω}-nitro-L-arginine methyl ester (L-NAME), 3-[3-cholamidopropyl)dimetholammonio]-1-propane sulphonate (CHAPS) and methoxamine were purchased from Sigma (St. Louis, U.S.A.). U46619 (already dissolved in methyl acetate) was purchased from Cayman Chemical Company (Ann Arbor, U.S.A.). Endothelin-1 was purchased from Peninsula Laboratories (Belmont, U.S.A.). All agents were dissolved in phosphate-buffered saline (PBS), except for [$MePhe^7$]NKB and [β-Ala^8]NKA(4–10) which were dissolved in 10% tetramethylene sulphone (Aldrich) then diluted further with PBS. Indomethacin was dissolved in Trizma base (pH 7.4; 0.2 M, Sigma) and noradrenaline was prepared in ascorbic acid (Baker).

Statistics

Wilcoxon signed rank test was used for non-parametric paired data. Mann Whitney-U statistical test was used for non-parametric grouped data and Student's *t* test was used for parametric paired or grouped data. *P* values of 0.05 and lower were considered to be significant.

Results

Effect of indomethacin on the arterial and venous reactivities of the mesenteric bed of the guinea-pig

In preliminary experiments, as previously stated, the effect of indomethacin (5 μM) was tested against the vasoconstrictor properties of endothelin-1 (100 pmol, arterial side; 1000 pmol, venous side) when perfused at a flow of 6 ml min^{-1}, the basal perfusion pressure was 19.9±1.3 mmHg on the arterial side and 10.4±1 mmHg on the venous side. A 30-min infusion of indomethacin significantly increased the perfusion pressure on both the arterial and venous sides (31.0±1.8 mmHg, arterial side; 21.5± 1.8 mmHg, venous side; $n=4$, $P<0.01$). Furthermore, the vasopressor effect of endothelin-1 on both sides of the mesenteric vasculature was markedly potentiated by indomethacin (arterial side, control: 2.0±0.7 mmHg; in presence of indomethacin: 4.6±0.2 mmHg; $n=4$, $P<0.01$), as well as the venous side (control: 1.9±0.4; in presence of indomethacin: 5.2±0.9; $n=4$, $P<0.05$). In order to avoid interference of vasodilator prostanoids in this system, indomethacin (5 μM) was subsequently added to the Krebs solution for all other experiments.

Effect of changes in perfusion flow in the arterial and venous reactivity of the mesenteric bed

The basal perfusion pressures of arterial and venous sides of the mesenteric vasculature were 3.6±0.4 mmHg ($n=32$) and 1.6±0.3 mmHg ($n=17$), respectively, when perfused at 2 ml min^{-1}. These pressures increased by 13.7±0.9 mmHg ($n=26$) and 8.8±0.7 mmHg ($n=17$), respectively, when the perfusion flow rate was increased to 6 ml min^{-1} and by 27.7±3.7 mmHg ($n=3$) and 36.5±5.4 mmHg ($n=3$), respectively, when further increased to 9 ml min^{-1}.

In the mesenteric beds which were subjected to a basal perfusion flow of 2 ml min^{-1}, methoxamine (200 μM) and U46619 (4.2 μM) induced a non-significant increase of perfusion pressure in the arterial and venous mesenteric vasculature, respectively. At an increase flow of 6 ml min^{-1}, methoxamine (200 μM, arterial side) and U46619 (4.2 μM, venous side) increased the perfusion pressures by 10.1±1.4 mmHg ($n=32$) and 2.8±0.3 mmHg ($n=17$), respectively. Furthermore, an increase of perfusion pressure to 9 ml min^{-1} did not further increase the responsiveness of the mesenteric vasculature to the same vasoconstrictors (7.2±0.7, arterial side; 2.53±0.4 mmHg, venous side, $n=3$), when compared to vessels perfused at 6 ml min^{-1}.

Effect of selective neurokinin analogues on the precontracted arterial and venous mesenteric vasculatures of the guinea-pig

In preconstricted preparations, acetylcholine (500 pmol) induced a transient vasodilatation of the arterial vessels and sodium nitroprusside (1000 pmol) a vasodilatation of the venous vasculature (Figure 1a and b). The NK_1 receptor-selective agonist, [Sar^9,Met(O$_2$)^{11}]SP (1 to 1000 pmol), induced a dose-dependent vasodilatation of the arterial mesenteric vasculature (ED_{50}: 20.2 pmol), whereas the NK_2 receptor agonist, [β-Ala^8]NKA(4–10) (1 to 1000 pmol), was inactive. The NK_3 receptor agonist, [MePhe^7]NKB (1 to 1000 pmol), caused a small arterial vasodilatation only at high doses (Figure 1c). The neurokinin analogues were without effect on the venous vessels (Figure 1b).

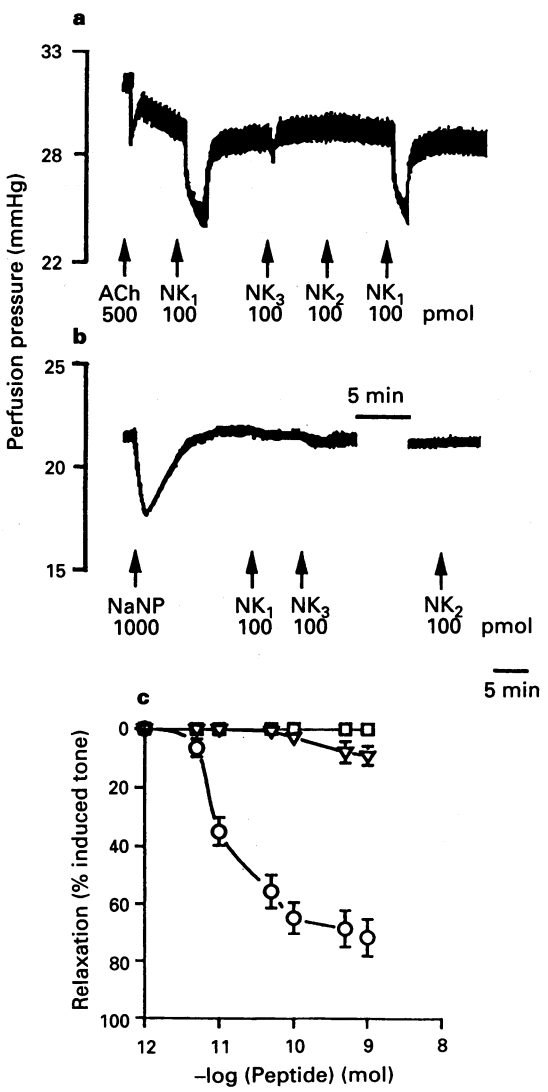

Figure 1 Typical traces illustrating the effect of acetylcholine (ACh), sodium nitroprusside (NaNP), [Sar^9,Met(O$_2$)^{11}]SP (NK_1), [MePhe^7]NKB (NK_3) and [β-Ala^8]NKA(4–10) (NK_2) on perfusion pressures within the arterial (a) and venous (b) vasculature precontracted, respectively, with methoxamine (200 μM) and U46619 (4.2 μM). (c) Dose-response curves of [Sar^9,Met(O$_2$)^{11}]SP (○), [MePhe^7]NKB (▽) and [β-Ala^8]NKA(4–10) (□) in the arterial mesenteric bed of the guinea-pig precontracted with methoxamine (200 μM). Each point with a bar represents the mean±s.e.mean of 4 to 19 experiments.

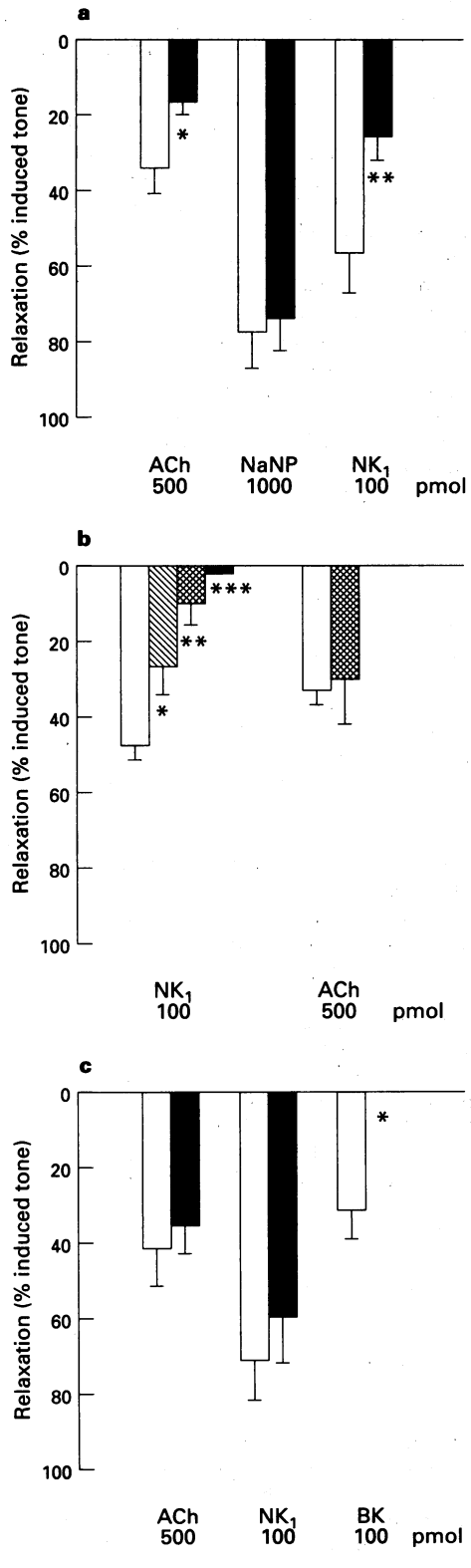

Figure 2 Vasodilatations induced by acetylcholine (ACh), sodium nitroprusside (NaNP), [Sar^9,Met(O$_2$)^{11}]SP (NK_1) or bradykinin (BK) in the arterial mesenteric vasculature of the guinea-pig precontracted with methoxamine (200 μM) before (open columns) or after treatment (a) with L-NAME (200 μM, solid columns); (b) before (open columns) or after treatment with CP-99,994 0.005 μM (hatched column), 0.05 μM (cross-hatched column) and 0.5 μM (solid column) and (c) before or after treatment with Hoe 140 (0.1 μM, solid columns). Each column represents the mean±s.e.mean of 4 to 20 determinations. $*P<0.05$, $**P<0.01$, $***P<0.001$ when compared to control values (open columns).

Effect of L-NAME, CP-99,994 and Hoe 140 on the vasodilatations induced by [Sar^9,Met(O$_2$)^{11}]SP

Acetylcholine or [Sar^9,Met(O$_2$)^{11}]SP induced vasodilatations in the arterial mesenteric vasculature precontracted with methoxamine which were reduced by L-NAME (200 μM) (acetylcholine, 500 pmol, reduced by 53%, $n=6$, $P<0.05$, [Sar^9,Met(O$_2$)^{11}]SP, 100 pmol, reduced by 54%, $n=7$, $P<0.01$) (Figure 2a). Vasodilatations induced by sodium nitroprusside (1000 pmol) were unaffected by L-NAME ($n=6$). L-NAME alone increased the perfusion pressure of the precontracted arterial side by 23.2 ± 5.5 mmHg ($n=7$) without affecting the response to sodium nitroprusside (Figure 2a). CP-99,994 (0.005 to 0.5 μM), an antagonist of NK$_1$ receptors (McLean *et al.*, 1993), blocked vasodilatations induced by [Sar^9,Met(O$_2$)^{11}]SP (100 pmol) with an IC$_{50}$ of 1.87×10^{-8} M. CP-99,994 (0.05 μM) did not affect vasodilatations induced by acetylcholine (500 pmol) (Figure 2b). Finally, Hoe 140 (0.1 μM), an antagonist of B$_2$ receptors (Wirth *et al.*, 1991; Rhaleb *et al.*, 1991), blocked vasodilatations induced by bradykinin (100 pmol) but was without effect on those induced by acetylcholine (500 pmol) or [Sar^9,Met(O$_2$)^{11}]SP (100 pmol) (Figure 2c).

Effect of bradykinin on the precontracted and non-precontracted arterial and venous mesenteric vasculatures

Bradykinin (0.1 to 100 pmol) induced dose-dependent vasodilatations of the precontracted arterial and venous vasculatures, whereas a higher dose of the same peptide (1000 pmol) induced a biphasic response, a transient vasoconstriction followed by a vasodilatation (Figures 3a, b, and 4a,b). In non-precontracted preparations, bradykinin induced dose-dependent vasoconstrictions of the arterial vessels and a much weaker effect on the venous vasculature (Figures 3c and d). In another series of experiments, treatment with a mixture of antagonists (atropine, methysergide and diphenhydramine, 1 μM, 20 min) did not reduce the contractile response of the arterial mesentery induced by bradykinin (1000 pmol) (control: 12.5 ± 1.7 mmHg; in presence of antagonists: 16.9 ± 2.6 mmHg, $n=5$).

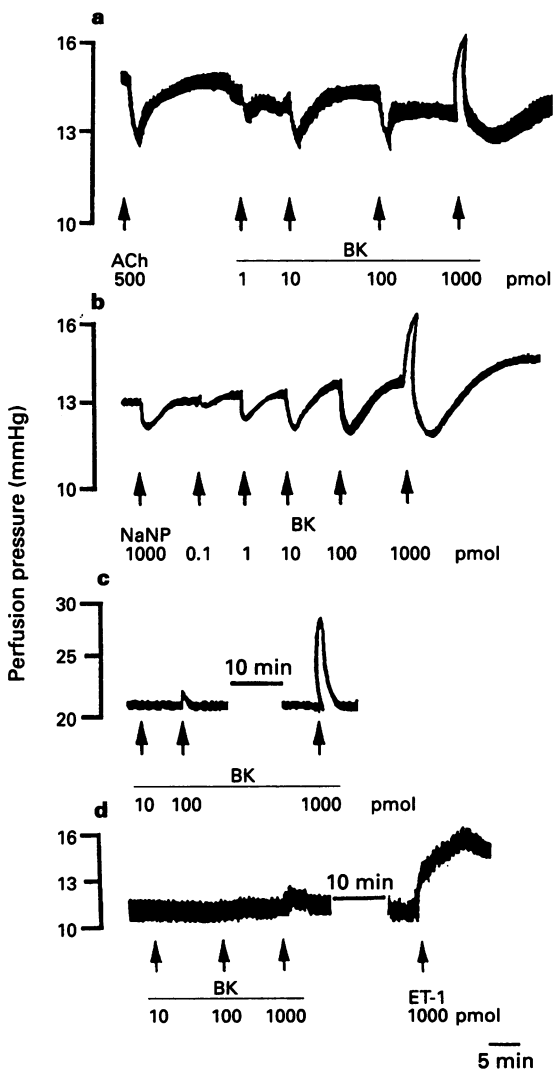

Figure 3 Typical traces illustrating the effects of acetylcholine (ACh), sodium nitroprusside (NaNP), bradykinin (BK) and endothelin-1 (ET-1) on the arterial (a) and venous (b) vasculatures precontracted respectively with methoxamine (200 μM) and U46619 (4.2 μM) and of BK on the non-precontracted arterial (c) and venous (d) vasculatures. Traces are representative of 5 to 11 experiments.

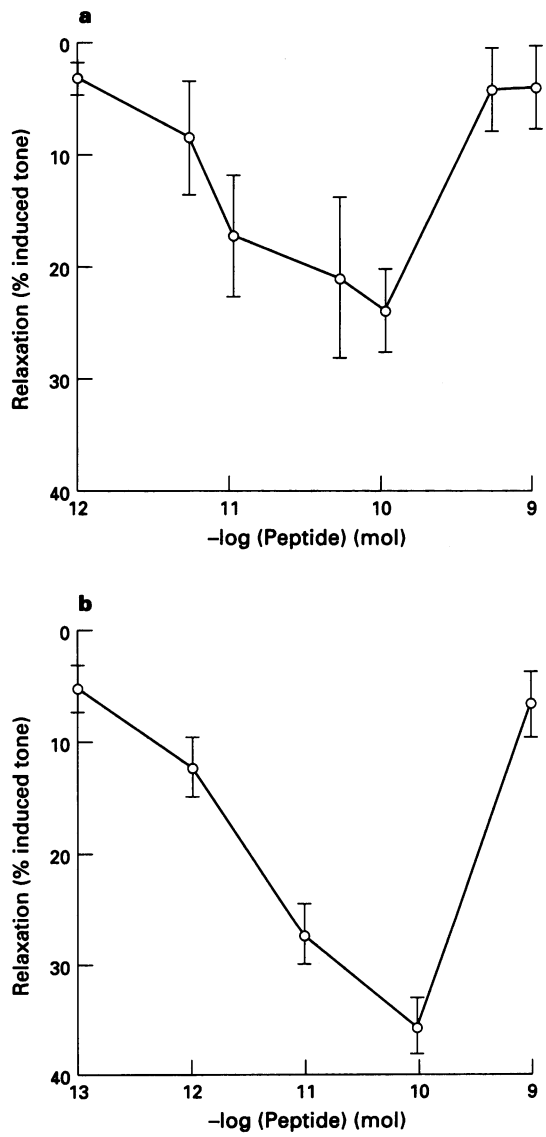

Figure 4 Dose-response curves of bradykinin (BK) on the arterial (a) or venous (b) mesenteric vasculature of the guinea-pig precontracted, respectively, with methoxamine (200 μM) or U46619 (4.2 μM). Each point represents the mean ± s.e.mean of more than 4 determinations.

Effect of Hoe 140, [Leu^8]desArg^9-BK and L-NAME on the response of bradykinin on the non-precontracted and precontracted arterial mesenteric vasculature

Hoe 140 (0.001 to 0.1 μM), an antagonist of B_2 receptors, reduced in a concentration-dependent fashion, the vasoconstriction of the non-precontracted arterial vasculature induced by bradykinin and the IC_{50} of the antagonist was established at 3.8×10^{-8} M (Figure 5a). Vasoconstrictions induced by endothelin-1 (100 pmol) were unaffected by (0.1 μM) Hoe 140 or [Leu^8]desArg^9-BK, an antagonist of B_1 receptors, (Regoli & Barabé, 1980). Hoe 140 (0.1 μM) also completely blocked vasodilatations of the precontracted arterial vessels induced by bradykinin (100 pmol), as well as the biphasic effect of bradykinin seen at a dose of 1000 pmol, without affecting responses to acetylcholine (500 pmol, results not shown). [Leu^8]desArg^9-BK (0.1 μM) did not affect the vasodilator properties of bradykinin (100 pmol) (control: 36.8±9.3%, in presence of [Leu^8]desArg^9-BK: 28.4±10.5%, $n=5$). L-NAME also abolished ($n=4$, $P<0.05$) vasodilatations of the precontracted arterial vessels induced by bradykinin (50 pmol) without affecting those induced by sodium nitroprusside (1000 pmol, Figure 5c).

L-NAME (200 μM) increased basal pressure (control: 17.3±1.3; in presence of L-NAME: 23.5±1.6 mmHg; $n=4$, $P<0.05$) and potentiated approximately by 4 fold the bradykinin-induced vasoconstrictions of the non-precontracted arterial vessels as well as by 10 fold the response of the same preparation to endothelin-1 (100 pmol) (Figure 5b).

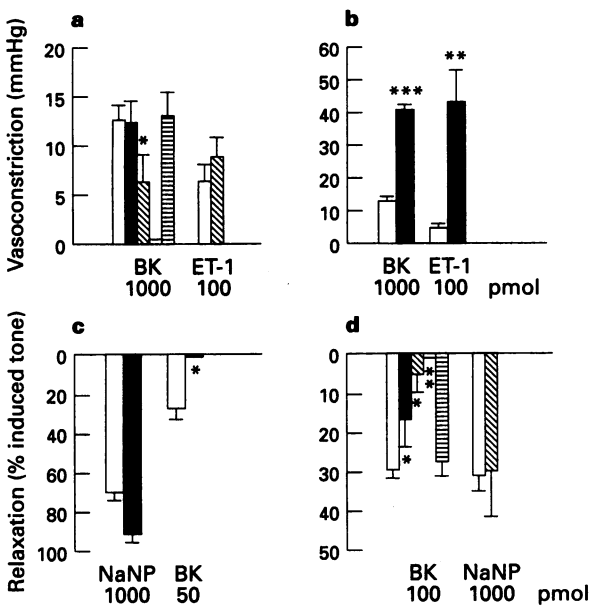

Figure 5 Effects of Hoe 140 and [Leu^8]desArg^9-BK on the responses of the arterial and venous mesenteric circulation and N^{ω}-nitro-L-arginine methyl ester (L-NAME) in the arterial vasculature of the guinea-pig. (a) Arterial constriction induced by bradykinin (BK) or endothelin-1 (ET-1) before (open columns) or after treatment with Hoe 140, 0.001 μM (solid column), 0.01 μM (hatched column) or 0.1 μM (cross-hatched column, $P<0.01$) or [Leu^8]desArg^9-BK 0.1 μM (horizontally lined column). (b) Effect of L-NAME (200 μM, solid columns) on arterial constriction induced by BK or ET-1. Control responses shown in open columns. (c) Effect of L-NAME (200 μM, solid columns) on vasodilatations (control, open columns) by BK or sodium nitroprusside (NaNP) on the precontracted arterial vessels. (d) Effects of Hoe 140 and [Leu^8]desArg^{9}BK at the same concentrations as depicted in (a) on the venodilatations induced by BK (100 pmol) or NaNP (1000 pmol). Each column represents the mean±s.e.mean of 4 to 40 determinations. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Effect of Hoe 140, [Leu^8]desArg^9-BK and L-NAME on responses to bradykinin in the precontracted or non precontracted venous mesenteric vasculature

In the precontracted venous vessels, Hoe 140 (0.001 to 0.1 μM) abolished venodilatations induced by bradykinin (100 pmol) with an IC_{50} of 1.63×10^{-8} M, yet the same antagonist did not inhibit, at 0.01 μM, the responses of the preparation to sodium nitroprusside (1000 pmol). [Leu^8]desArg^9-BK (0.1 μM) did not affect any of the responses to the agonists (Figure 5d). Hoe 140 (0.1 μM) also abolished the biphasic effect of bradykinin seen at a dose of 1000 pmol; this response was unaffected by [Leu^8]-desArg^9-BK (0.1 μM) (data not shown). In addition, L-NAME (200 μM) markedly reduced by 75% the vasodilatation induced by bradykinin (100 pmol) (control: 50.4±9.0%; in presence of L-NAME: 12.5±5.6%, $n=8$, $P<0.01$) without reducing the response to sodium nitroprusside (1000 pmol) (control: 33.5±10.1%; in presence of L-NAME: 43.9±6.5%, $n=8$).

In non-precontracted vasculatures, treatment with L-NAME (200 μM) did not significantly increase the perfusion pressure (control: 10.4±1.0 mmHg; in presence of L-NAME: 12.4±0.7 mmHg, $n=4$). The same treatment enhanced the contractile response to bradykinin (1000 pmol) control: 0.5±0.2 mmHg, in presence of L-NAME: 1.8±0.3 mmHg, $n=7$, $P<0.05$) and endothelin-1 (1000 pmol) (control: 4.7±1.2 mmHg, in presence of L-NAME: 7.3±2.9 mmHg, $n=6$, $P<0.05$).

CHAPS abolishes the vasodilator properties of BK in the arterial and venous mesenteric vasculatures

In a separate series of experiments, a subsequent treatment of the arterial and venous mesenteric vasculatures with a 30 s infusion of CHAPS (20 mM) abolished the vasodilator properties of bradykinin (arterial side; control: 31.0±7.7; in presence of CHAPS: 0 mmHg, $n=3$, $P<0.01$) (venous side; control: 28.9±2.1; in presence of CHAPS: 0 mmHg, $n=3$, $P<0.01$). In contrast, the same detergent did not affect the vasodilator properties of sodium nitroprusside on either side of the mesenteric vasculature (results not shown).

Lack of arterio-venous shunts in the non-precontracted arterial and venous mesenteric vasculatures

The non-precontracted arterial and venous mesenteric vasculatures responded to angiotensin II and noradrenaline by vasoconstrictions (as also previously shown with endothelin-1) on both sides of the guinea-pig mesenteric circulation (Figure 6). In general, the venous vasculature was less responsive to these agonists than the arterial counterpart. Finally, bolus injections of the above-mentioned agonists into the arterial or venous vasculatures did not influence tone of the alternate portion of the vasculature.

Discussion

We have characterized the vasoactive properties of neurokinins and kinins in the arterial and venous mesenteric beds of the guinea-pig. Our results show that both the arterial and venous mesenteric vasculatures are highly sensitive to bradykinin which acts through B_2 receptor activation, whereas only the arterial vessels exhibit responses to NK_1 receptor activation, namely endothelium-dependent relaxation. In a separate series of experiments, CHAPS also abolished the arterial vasodilator effects of [Sar^9,Met(O$_2$)^{11}]SP in the guinea-pig mesenteric bed (results not shown).

The presence of NK_1 receptors mediating vasodilatations within the arterial mesenteric vasculature of the guinea-pig was confirmed by the use of CP-99,994 which abolished, at a low concentration (0.05 μM), the response to the selective NK_1 agonist, [Sar^9,Met(O$_2$)^{11}]SP. The specificity of action of the antagonist was confirmed by the lack of effect of CP-99,994 on

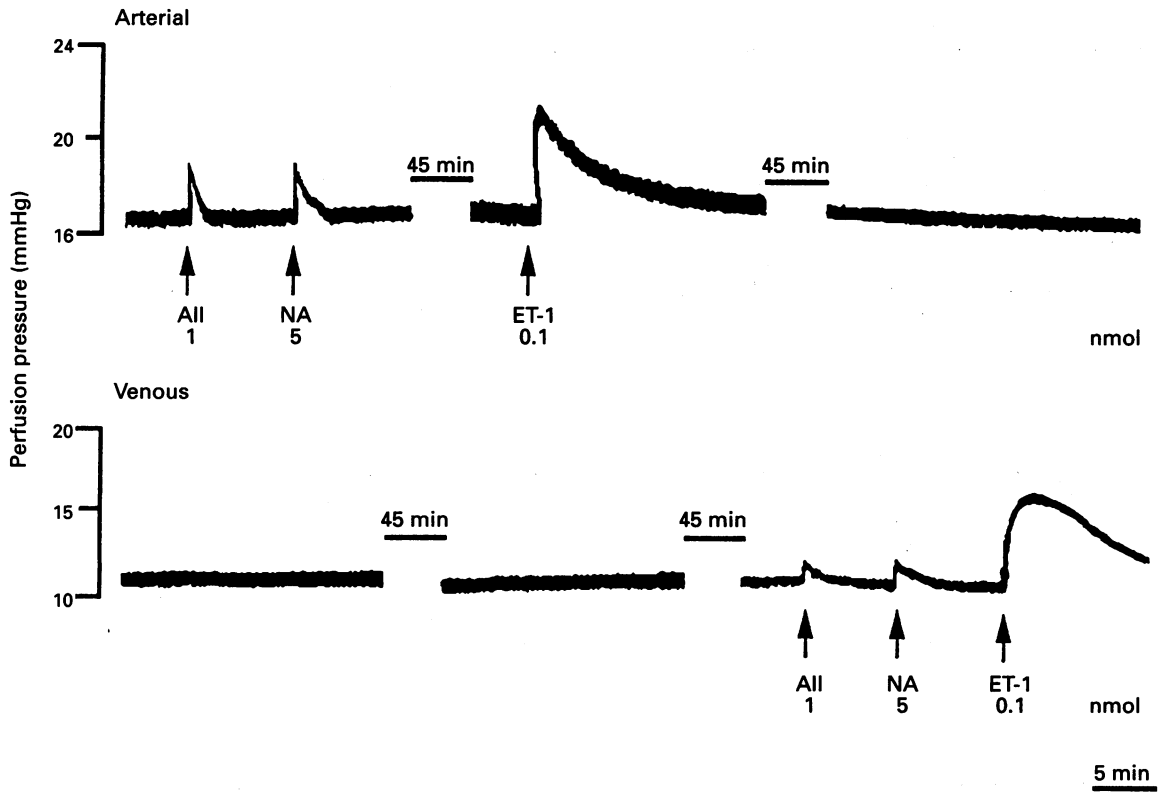

Figure 6 Typical traces illustrating the effect of angiotensin II (AII), noradrenaline (NA) and endothelin-1 (ET-1) on the non-precontracted arterial and venous vasculatures (lack of veno-arterial shunts following AII administration is shown). Traces are representative of 5 experiments.

vasodilatations induced by acetylcholine in the same vascular bed. It is interesting that the guinea-pig arterial mesenteric vasculature responded to NK_1 activation, because the rat counterpart is relatively insensitive to neurokinins. For instance, natural neurokinins produce little effect in the arterial vessels (Kawasaki *et al.*, 1988) and high doses of [Sar^9,Met$(O_2)^{11}$]SP do not relax either the arterial or the venous vasculature of the rat (D'Orléans-Juste *et al.*, 1991). The second difference between guinea-pig and rat mesenteric vasculatures is that the NK_1, NK_2 and NK_3 agonists are inactive in the venous vessels of preparations from the former species, while [MePhe^7]NKB causes constrictions of the venous mesenteric vasculature of the rat via activation of NK_3 receptors (D'Orléans-Juste *et al.*, 1991).

In contrast to the neurokinins, bradykinin-induced vasodilatations on both sides of the guinea-pig mesenteric vasculature were similar to those seen in preparations from the rat (Warner, 1990). Furthermore, as shown in the rat mesenteric vasculature (Warner, 1990), the vasodilatations induced by the kinins in the guinea-pig vasculature were sensitive to an inhibitor of nitric oxide synthase, L-NAME, indicating that they were mediated by nitric oxide and endothelium-dependent in nature, as confirmed with CHAPS.

Vasodilatations induced by bradykinin in the venous and arterial beds were mediated via the activation of B_2 receptors, for they were selectively antagonized by the selective B_2 receptor antagonist, Hoe 140, but unaffected by the selective B_1 antagonist, [Leu^8]desArg^9-bradykinin. This finding supports the interpretation that the vascular responses to bradykinin in the guinea-pig are mediated exclusively by B_2 receptors (Hall, 1992). The apparent affinity of bradykinin (estimated as ED_{50}) for inducing vasodilatation was 5.5 pmol on the arterial side and 1.9 pmol on the venous side, indicating that the B_2 receptor has the same characteristics in the arterial and venous endothelium.

It is interesting that the vasoconstrictions induced by the higher doses of bradykinin, as established by their sensitivity to Hoe 140, were also mediated by B_2 receptor activation. These receptors are most probably located on the smooth muscle of the mesenteric arterial and venous vasculatures, as has been suggested from studies on guinea-pig isolated mesenteric veins (Gaudreau *et al.*, 1981). The precise characterization of B_2 receptors and their location in the smooth muscle of the guinea-pig mesenteric vasculature remain to be determined and will be further investigated in a subsequent study.

Under what we suggest to be optimal perfusion flow rates for both sides of the guinea-pig mesenteric vasculature, we did not obtain increases in perfusion pressure following the application of methoxamine to the arterial side and of U46619 to the venous side nearly as great as those obtained in the rat mesenteric vasculature (Warner, 1990; D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992; 1994). The weaker vasoconstrictor responses of the guinea-pig versus the rat mesenteric vasculature were particularly evident on the venous side. Indeed, we were unable to obtain increases in venous perfusion pressure by more than 6 or 7 mmHg. However, one has to take into account that the normotensive conscious guinea-pig has a mean arterial blood pressure of about 50 mmHg (Coyle *et al.*, 1988), whereas in the rat, the mean arterial pressure is approximately 100 mmHg (Gardiner *et al.*, 1992).

Hence, the lower basal perfusion pressure obtained in the arterial and venous mesenteric vasculatures of the guinea-pig, as opposed to the rat, may be explained by the lower mean arterial blood pressure encountered in the guinea-pig. Nonetheless, endothelium-dependent or independent vasodilatations with bradykinin and sodium nitroprusside, respectively, could be measured in the guinea-pig venous mesenteric bed.

Enhancement by L-NAME of vascular tone in preparations at basal perfusion pressure or precontracted shows the important contribution of flow-induced release of nitric oxide at least in the arterial mesentery. However, we showed that blockade of EDRF release by L-NAME did not affect the response of the preparation to an endothelium-independent vasodilator, sodium nitroprusside, suggesting that following inhibition of the nitric oxide synthase, vascular reactivity remained unaffected.

Finally, in addition to BK and neurokinins, we have studied the pharmacological properties of angiotensin II and endothelin-1 in the arterial and venous mesenteric beds of the guinea-pig. However, further studies will be necessary to characterize fully the responses to these vasoactive peptides. It is worthy of notice that vasodilator prostanoids appear to act as important modulators in the guinea-pig mesenteric vasculature, as illustrated by the marked potentiation of the response to ET-1 by indomethacin. It is also of interest that artero-venous shunts have a negligible role in mediating the vasoactive effects of the various agents studied. Overall, the development and use of this new model may give us some insights on interspecies differences in the response to various agonists and antagonists in the rat and guinea-pig pre and post-capillary mesenteric vasculatures.

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