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Short communication

Interactions of pro-inflammatory and vasoactive mediators with nitric oxide in the regulation of rat vascular permeability during laparotomy

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Abstract

Inhibition of constitutive nitric oxide (NO) synthases by administration of N^G-nitro-L-arginine methyl ester (L-NAME) during abdominal laparotomy provokes extensive vascular leakage in the rat gastrointestinal tract, assessed by the extravasation of [^{125}I]human serum albumin. In the present study, the role of vasoactive or neutrophil-derived pro-inflammatory mediators in this process has been investigated. Administration of the thromboxane synthase inhibitor, 1-benzyl-imidazole (BZI, 25–50 mg kg^{-1}, s.c.), the platelet-activating factor (PAF) receptor antagonist, 3-[4-(2-chlorophenyl)-9-methyl-6*H*-thienol-[3,2-f][1,2,4]-triazolo-[4,3-a][1,4]-diazepine-2-yl]-1-(4-morpholynil)-1-propionate (WEB 2086; 0.5–1 mg kg^{-1}, s.c.), the 5-lipoxygenase synthase inhibitor, *N*-(4-benzyloxybenzyl)-acetohydroxamic acid (BW A137C; 4–20 mg kg^{-1}, s.c.) or the vasopressin pressor receptor antagonist ([Mca^1,Tyr(Me)^2,Arg^8]vasopressin/Manning peptide; 0.01–0.2 μg kg^{-1}, s.c.) dose-dependently reduced the intestinal plasma leakage provoked by L-NAME (5 mg kg^{-1}, s.c.), following a 5-cm abdominal laparotomy in anaesthetised rats. These findings suggest that constitutive NO synthase effectively counteracts the damaging actions on microvascular integrity of mediators, including thromboxanes, PAF, leukotrienes and vasopressin, released during surgical intervention.

Keywords: Nitric oxide (NO); Vascular permeability; Vascular endothelium; Neutrophil granulocytes; Platelet-activating factor (PAF); Thromboxanes; Leukotrienes; Vasopressin; Surgery

1. Introduction

During major surgical operations, a reduction in circulating plasma volume can occur, following increased plasma extravasation, which develops without significant bleeding (Krakelund, 1971; Akerström and Lisander, 1991). However, few experimental or clinical studies have explored the mechanism underlying this phenomenon (Akerström and Lisander, 1991; László and Whittle, 1999; László et al., 1999).

Nitric oxide (NO), formed continuously in the vascular endothelium by the constitutive NO synthase, eNOS, plays a key role in the maintenance of vascular integrity (Moncada and Higgs, 1995). NO attenuates the adherence and immigration of leukocytes in the vascular endothelium (Kubes et al., 1991), a process of key importance in the generation of vascular endothelial dysfunction, vascular congestion and inflammation (Wedmore and Williams, 1981). Indeed, inhibition of the constitutive NO synthase, eNOS, leads to neutrophil adhesion and the elevation of vascular permeability, particularly in surgically prepared models (Kubes et al., 1991; Kubes and Granger, 1992; Arndt et al., 1993).

In recent studies, constitutively formed NO has been suggested to play a beneficial role against widespread plasma loss, observed during surgical operation, through the maintenance of microvascular integrity (László and Whittle, 1999; László et al., 1999). Thus, midline abdominal laparotomy and anaesthesia alone, or administration of

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the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), to anaesthetised animals alone, did not affect vascular permeability. In contrast, when L-NAME was administered to laparotomized animals, a generalised increase of albumin leakage developed in intestinal organs as well as in the lungs and kidneys (László and Whittle, 1999). A reduction in the number of circulating neutrophils attenuated this vascular leakage provoked by L-NAME and laparotomy in all of the organs investigated, which suggests an involvement of neutrophil-derived pro-inflammatory mediators in this surgical plasma extravasation (László and Whittle, 1999).

In previous studies, a role for the neutrophil-derived pro-inflammatory mediators thromboxanes, platelet-activating factor (PAF) and leukotrienes, on the development of vascular leakage provoked by the acute administration of L-NAME following bacterial endotoxin challenge, has been established (László and Whittle, 1995; László et al., 1994). In the present work, the involvement of such mediators in responding to L-NAME following midline abdominal laparotomy has been explored using specific antagonists and synthase inhibitors. In addition, using a specific vasopressin pressor receptor antagonist, the actions of endogenous vasopressin, which is known to be released during surgical interventions (Melville et al., 1985), have been evaluated.

2. Materials and methods

2.1. Surgical manipulation

Male Wistar rats (225–275 g) were starved overnight, but were allowed free access to water. In the basal control group, the treatments were performed under transient halothane anaesthesia from which the animals had completely recovered within 2 min. Autopsy in this surgically unoperated group was performed under halothane anaesthesia. In all other groups (using pentobarbitone-anaesthetised animals), a 5-cm long midline laparotomy in the abdominal wall was performed without significant bleeding. Rats were tracheotomized and a gauze pad moistened with saline was placed over the incision. At the beginning of the experiment, all groups were administered [^{125}I]human serum albumin (2 μCi kg^{-1}, i.v.) via a needle inserted into the tail vein for 3–4 s. Autopsy was performed 1 h later.

2.2. Plasma leakage

As a measure of vascular endothelial permeability, leakage of [^{125}I]human serum albumin into tissue was determined in segments of the jejunum and colon. Blood were collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and were centrifuged (10,000 × g, 10 min, 4°C). The [^{125}I]human serum albumin content of the plasma and of segments of tissues was detected in a gamma spectrometer (Nuclear Enterprises NE 1600), and the albumin content in tissues was calculated.

The resting value for albumin accumulation was taken as the mean of the data of a group of basal control animals. In each experiment, and for each procedure, this basal control mean value was calculated and subtracted from the value from each of the animals in each treatment group. The data were expressed as changes in albumin accumulation (Δplasma leakage, in microliter plasma per gram tissue) corrected for intravascular volume (László et al., 1994).

2.2.1. Intravascular volume

Changes in intravascular volume in jejunal and colonic tissues were determined in additional groups of rats by administering [^{125}I]human serum albumin (2 μCi kg^{-1}) intravenously via the tail vein 2 min before tissue removal, in all groups investigated. The tissue and plasma content of radiolabel were determined and the intravascular volume was expressed as microliter per gram tissue (László et al., 1994).

2.3. Effect of L-NAME and laparotomy on intestinal plasma leakage

In groups of rats, L-NAME (5 mg kg^{-1}, s.c.) was injected concurrently with [^{125}I]human serum albumin. Plasma leakage in the jejunum and colon was evaluated after 1 h. The dose of L-NAME and the timing of the experiment were based on the results of our previous study (László and Whittle, 1999).

2.4. Effect of pro-inflammatory and vasoactive mediators on plasma leakage provoked by L-NAME and laparotomy

In laparotomized and L-NAME (5 mg kg^{-1}, s.c.)-treated rats, the thromboxane synthase inhibitor, 1-benzyl-imidazole (BZI; 25–50 mg kg^{-1}), the PAF receptor antagonist, (3-[4-(2-chlorophenyl)-9-methyl-6*H*-thienol-[3,2-f][1,2,4]-triazolo-[4,3-a][1,4]-diazepine-2-yl]-1-(4-morpholynil)-1-propionate (WEB 2086; 0.5–1 mg kg^{-1}), the 5-lipoxygenase inhibitor, *N*-(4-benzyloxybenzyl)-acetohydroxamic acid (BW A137C; 4–20 mg kg^{-1}) or the vasopressin pressor receptor antagonist ([Mca^1,Tyr(Me)^2, Arg^8]vasopressin/Manning peptide; 0.01–0.2 μg kg^{-1}) was injected s.c., 15 min before laparotomy and L-NAME administration. Plasma leakage in jejunal and colonic tissues was determined 1 h after laparotomy. All the compounds were dissolved in saline immediately before the experimentation. The doses, timing and route of administration of drugs were established in our previous studies

(László et al., 1991, 1994; László and Whittle, 1995, 1999).

2.5. Chemicals

[^{125}I]human serum albumin was obtained from Amersham International (UK). BZI and BW A137C originated from the Wellcome Research Laboratories, Beckenham, UK. WEB 2086 was supplied by the Boehringer Ingelheim, Germany. The vasopressin antagonist was acquired from Bachem, Germany. All the other compounds were from Sigma (Poole, Dorset, UK).

2.6. Statistics

The data are expressed as mean ± SEM from (n) rats per experimental group. For statistical comparisons, analysis of variance with the Bonferroni test was utilised, where $P < 0.05$ was taken as significant.

3. Results

3.1. Intestinal albumin leakage provoked by L-NAME during laparotomy

No significant intestinal albumin leakage could be observed when L-NAME (5 mg kg^{-1}, s.c.) was administered alone into the anaesthesized non-laparotomized rat after 1 h ($\Delta 2 \pm 4$ and $\Delta 6 \pm 3$ μl g^{-1} tissue in the jejunum and colon, respectively; $n = 7$). Laparotomy alone did not provoke significant jejunal or colonic albumin leakage over the 1-h study period ($\Delta 5 \pm 7$ and $\Delta 1 \pm 7$ μl g^{-1} tissue in the jejunum and colon, respectively; $n = 8$). In contrast, when L-NAME was administered concurrently with the laparotomy procedures, significant albumin leakage occurred in jejunal and colonic tissues ($\Delta 82 \pm 3$ and $\Delta 61 \pm 5$ μl g^{-1} tissue, respectively; $n = 9$, $P < 0.005$), as demonstrated in Fig. 1.

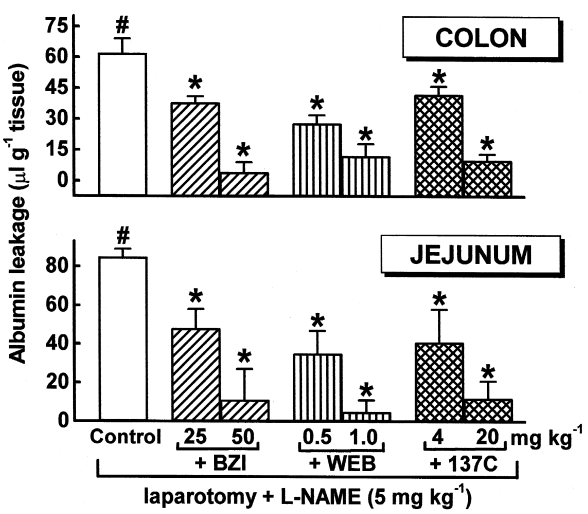

Fig. 1. Jejunal and colonic albumin leakage provoked by L-NAME (5 mg kg^{-1}, s.c.) in the laparotomized rat over 1 h. The effects of pretreatment (15 min) with a thromboxane synthase inhibitor (BZI, 25–50 mg kg^{-1}, s.c.), with a PAF receptor antagonist, WEB 2086 (WEB, 0.5–1.0 mg kg^{-1}, s.c.), or with a leukotriene synthase inhibitor, BW A137C (137C, 4–20 mg kg^{-1}, s.c.) are shown. Data are expressed as the mean ± SEM, where (n) is minimum five rats for each group, and where statistical significance is given as $^{\#}P < 0.05$ compared to conscious untreated animals; $^{*}P < 0.05$ compared to L-NAME plus laparotomy (control) groups.

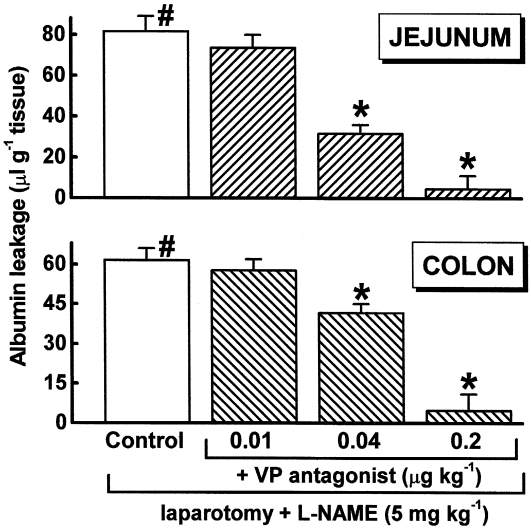

Fig. 2. Dose-dependent attenuation of L-NAME (5 mg kg^{-1}, s.c.)-provoked rat jejunal and colonic albumin leakage in laparotomized rats over 1 h, by the pretreatment (15 min) with the vasopressin pressor receptor antagonist (VP antagonist, 0.01–0.2 μg kg^{-1}, s.c.). Data are expressed as the mean ± SEM, where (n) is minimum five rats for each group, and where statistical significance is given as $^{\#}P < 0.05$ compared to conscious untreated animals; $^{*}P < 0.05$ compared to L-NAME plus laparotomy (control) groups.

3.2. Role of pro-inflammatory and vasoactive mediators in intestinal plasma leakage provoked by L-NAME and laparotomy

Pretreatment (15 min) with the thromboxane synthase inhibitor (BZI, 25–50 mg kg^{-1}, s.c.), the PAF receptor antagonist (WEB 2086, 0.5–1 mg kg^{-1}, s.c.) or a 5-lipoxygenase inhibitor (BW A137C, 4–20 mg kg^{-1}, s.c.) dose-dependently attenuated the subsequent jejunal and colonic plasma leakage provoked by laparatomy and L-NAME, with a maximum reduction of 88 ± 15%, 97 ± 2% or 87 ± 6% respectively in the jejunum ($n = 5$–7; $P < 0.001$), as shown in Fig. 1.

Administration of the vasopressin pressor receptor antagonist, [Mca^{1},Tyr(Me)^{2},Arg^{8}]vasopressin (0.01–0.2 μg kg^{-1}, s.c., 15 min before laparotomy and L-NAME), decreased jejunal and colonic albumin extravasation induced by L-NAME in the laparotomized rat, with a maximum reduction of 98 ± 1% in the jejunum ($n = 6$; $P < 0.001$), as shown in Fig. 2.

4. Discussion

In the present study, administration of the NO synthase inhibitor, L-NAME provoked albumin extravasation in the rat intestinal tract following laparotomy, confirming previous observations (László and Whittle, 1999; László et al., 1999). Other workers had earlier demonstrated that L-NAME could induce an increase in vascular permeability in surgically manipulated models (Kubes and Granger, 1992; Filep and Földes-Filep, 1993). These data show that constitutive NO plays a significant beneficial role in the maintenance of microvascular integrity during and following such surgical operations.

Inhibition of NO synthase enhances the adhesion of leukocytes to the vascular endothelium in surgically prepared vascular beds (Kubes et al., 1991; Arndt et al., 1993). Adhesion of polymorphonuclear leukocytes to the vascular endothelium is well-established to play a crucial role in the increase of microvascular permeability (Wedmore and Williams, 1981; Bone, 1991), most likely by releasing neutrophil-derived pro-inflammatory mediators, such as thromboxanes, PAF and leukotrienes (Bone, 1991). Indeed, previous studies have demonstrated that administration of PAF receptor antagonists decreased L-NAME-induced vascular permeability in the post-operative period (Filep and Földes-Filep, 1993). Furthermore, treatment with 5-lipoxygenase inhibitors or PAF receptor antagonists attenuated L-NAME-provoked enhancement of adhesion of neutrophils to the vascular endothelium following surgical preparation (Arndt et al., 1993).

In previous studies, L-NAME has also been shown to enhance substantially the vascular leakage following the acute administration of bacterial endotoxin (László et al., 1994). This acute response was attenuated by PAF receptor antagonists, thromboxane synthase inhibitors or a 5-lipoxygenase inhibitor, BW A137C, suggesting a role for these neutrophil-derived mediators in this process (László et al., 1994; László and Whittle, 1995). Likewise, in the present study, the PAF receptor antagonist, WEB 2086, the thromboxane synthase inhibitor, BZI, or the 5-lipoxygenase inhibitor, BW A137C, significantly attenuated albumin extravasation. Thus, the release of the neutrophil-derived pro-inflammatory mediators PAF, thromboxanes and leukotrienes appears to participate in the increase of intestinal vascular permeability observed as a consequence of acute abdominal surgery following administration of L-NAME.

The release of the pituitary nonapeptide, vasopressin, into the general circulation during surgical operations has been described (Melville et al., 1985). It has been known that vasopressin can be released from the vascular endothelium (Burnstock, 1991). In addition, pathological levels of vasopressin can provoke vascular endothelial dysfunction via its pressor receptors (László et al., 1991). In the present study, administration of the vasopressin pressor receptor antagonist dose-dependently attenuated albumin leakage provoked by L-NAME in the laparotomized rat. Thus, endogenous vasopressin may also play a role in the development of the enhanced microvascular permeability during surgical intervention.

Our current studies suggest that the regulation of the maintenance of microvascular integrity during surgical operations involves complex interactions between endothelium- and neutrophil-dependent mechanisms. Thus, constitutively formed NO, most likely of endothelial origin, appears to play a significant pathophysiological role in preventing albumin extravasation in response to surgical challenge. Injurious mediators, such as the neutrophil-derived pro-inflammatory thromboxanes, PAF and leukotrienes, appear to be released following laparotomy, as does vasopressin; the latter of which may originate from the posterior pituitary or from the vascular endothelium. The present findings suggest that such mediators may interact either synergistically or in a sequential cascade to provoke the eventual microvascular dysfunction. The processes that stimulate such mediator release following minor surgery are as yet unknown, although the present work suggests that the release or actions of these factors can be modulated by endogenous NO.

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