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Nitric oxide modulates the gastrointestinal plasma extravasation following intraabdominal surgical manipulation in rats

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Abstract

The actions of nitric oxide (NO) on gastrointestinal plasma loss, assessed by the leakage of [^{125}I]human serum albumin, provoked by intraabdominal surgery and organ manipulation has been investigated in pentobarbitone-anaesthesized rats. Gentle manipulation (3 min) of the stomach or the small intestine following laparotomy leads to an increase in albumin extravasation in the stomach, duodenum, jejunum and colon over 1 h. Administration of the NO synthase inhibitors, N^G-nitro-L-arginine methyl ester (1–5 mg kg^{-1}, s.c.) and N^G-monomethyl-L-arginine (12.5–50 mg kg^{-1}, s.c.), provoked a further substantial elevation of gastrointestinal albumin extravasation in the surgically manipulated rat, but not in control rats. This effect could be prevented by the pretreatment (15 min) with L-arginine (300 mg kg^{-1}, s.c.) or by the concurrent infusion of the NO donor, *S*-nitroso-glutathione (5 μg kg^{-1} min^{-1}, i.v.). Endogenous NO, most likely formed by endothelial NO synthase, thus appears to maintain microvascular integrity during surgery and organ manipulation of the gastrointestinal tract.

Keywords: Nitric oxide (NO) endothelial; Vascular permeability; Gastrointestinal tract; Surgery; Plasma loss; Microcirculation

1. Introduction

Experimental and clinical studies have shown that a reduction in circulating plasma volume can occur during or following major surgical intervention (Jarnum, 1961; Krakelund, 1971; Robarts, 1979; Akerström and Lisander, 1991). However, there is no clear understanding of the mechanism of such plasma extravasation associated with surgical manipulation or the processes that regulate it (Akerström and Lisander, 1991).

Nitric oxide (NO), formed continuously in the vascular endothelium and in neuronal elements by the constitutive NO synthase (endothelial NO synthase and neuronal NO synthase, respectively), plays a key role in the maintenance of microvascular integrity under physiological circumstances (Moncada et al., 1991; Moncada and Higgs, 1995). However, under pathological conditions (such as following endotoxin or cytokine administration), the widespread expression of the inducible NO synthase can be detected in many cell types including the vascular endothelium, neutrophils, macrophages and intestinal epithelial cells which leads to the overproduction of NO (Moncada et al., 1991; Moncada and Higgs, 1995).

The expression of inducible NO synthase provokes microvascular leakage of albumin into gastrointestinal tissues following endotoxin administration (Boughton-Smith et al., 1993). Moreover, the selective inhibition of inducible NO synthase by the bisisothiourea derivative, 1400 W, protects the microvasculature against such plasma loss (László and Whittle, 1997). Thus, the uncontrolled production of NO under pathological conditions is detrimental towards the vascular endothelium, which may involve the generation of such tissue damaging radicals as the hydroxyl and peroxynitrite (Beckman et al., 1990; Hogg et al., 1992; Lipton et al., 1993).

In contrast, the physiological generation of NO by endothelial NO synthase has beneficial microcirculatory effects. Thus, inhibition of endothelial NO synthase by the administration of the nonselective NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA), provokes intestinal albumin leakage following challenge with low doses of endotoxin,

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under conditions where neither endotoxin nor NO synthase inhibitors alone affected microvascular albumin extravasation (László et al., 1994a). Furthermore, L-NMMA augmented gastrointestinal microvascular damage induced by high doses of endotoxin (Hutcheson et al., 1990). Constitutive NO synthase has a beneficial role during the initiation of intestinal inflammation, since its inhibition in the early phase of various inflammatory bowel disease models provokes or augments microvascular leakage (Kiss et al., 1997; László and Whittle, 1998).

Endogenous NO attenuates the adherence and immigration of leukocytes in the vascular endothelium (Kubes et al., 1991), actions that have key importance in the generation of vascular permeability and inflammation (Wedmore and Williams, 1981). Indeed, administration of inhibitors or antagonists of such neutrophil-derived mediators as platelet-activating factor, thromboxanes and leukotrines attenuated intestinal microvascular injury provoked by the inhibition of endothelial NO synthase in the early phase of experimental sepsis and intestinal inflammation (László et al., 1994b; László and Whittle, 1998).

In a recent study, following the administration of the NO synthase inhibitors, L-NAME or L-NMMA, abdominal laparotomy produced a significant elevation in microvascular leakage in the jejunum and colon over 1 h (László and Whittle, 1999). These NO synthase inhibitors had no such effect in conscious, anasthesized or skin-incised rats. In addition, no significant difference in albumin leakage and accumulation was observed in tissues from unoperated conscious or anaesthesized rats, or in anaesthesized rats with a skin-incision or abdominal laparotomy under resting conditions, showing that these minor surgical interventions alone do not provoke changes in microvascular permeability to albumin.

In many studies on the microcirculation, the tissues under investigation are exposed by laparotomy. Moreover, in such studies, the organs such as those of the gut, are further manipulated or exteriorised, to expose a suitable region for the observation. Thus, in the present study, we have now extended the findings with laparotomy alone, to evaluate the effects of abdominal surgery with gentle manipulation of the gastrointestinal organs on vascular permeability, and to study the effects of inhibitors of NO synthase.

2. Materials and methods

2.1. Surgical manipulation

Male Wistar rats (225–275 g) were fasted overnight, but allowed for free access to water. The animals were separated into two groups:

In the control group, the animals were deemed to be conscious for the majority of the experimentation period, since the treatments were performed under transient halothane anaesthesia from which the animals had completely recovered within 2 min. Autopsy in this group was performed under halothane anaesthesia within 1 min.

In the laparotomy and organ manipulated groups (termed surgical manipulation), the animals received pentobarbitone (60 mg kg^{-1}, i.p.) to induce anaesthesia and were tracheotomized. A 5-cm-long midline laparotomy in the abdominal wall was performed, without significant bleeding. In the manipulated group, part of the small bowel was exteriorised and gently handled for 3 min in a 5×5 cm gauze pad moistened in 37°C saline. The bowel was placed back into the abdominal cavity, and a gauze moistened with saline was placed over the incision for the protection against evaporation. Those rats who had significant bleeding were excluded from the study. In all of the anaesthetised groups, the body temperature was maintained on 36.5–37°C using a homeothermic control unit and underblanket (Harvard Instruments).

[^{125}I]human serum albumin (2 μCi kg^{-1}, i.v.) was administered via a needle inserted into the tail vein, and autopsy was performed 1 h later, i.e., the time-interval between the start of surgical procedures and autopsy was 60 min. The 1 h maximum timepoint was chosen to exclude the involvement of inducible NO synthase, that requires 2–3 h following challenge for expression, since this could modify vascular leakage and hence confound interpretation of the findings (Boughton-Smith et al., 1993).

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 stomach, duodenum, jejunum and colon. Blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and centrifuged ($10{,}000 \times g$, 10 min, 4°C). The [^{125}I]human serum albumin content of the plasma and segments of tissues was determined in a gamma-spectrometer (Nuclear Enterprises NE 1600) and the albumin content in tissues was calculated. The control value for albumin accumulation was taken as the mean of the data of a group of control unanaesthesized animals, which received albumin only, which reflects basal albumin movement into tissues. This basal control mean value was calculated and subtracted from the value from each of the animals in each experimental group. The data were expressed as changes in albumin accumulation (Δ plasma leakage, μl plasma g^{-1} tissue), corrected for intravascular volume as described previously (Boughton-Smith et al., 1993; László et al., 1994a).

2.3. Effect of L-NAME and L-NMMA on gastrointestinal plasma leakage

In a set of rats from each of the groups, L-NAME (1–5 mg kg^{-1}, s.c.) or L-NMMA (1–50 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. In a separate group, rats were pretreated with L-arginine (300 mg kg^{-1}, s.c.) 15 min before L-NAME (5 mg kg^{-1}, s.c.) administration, and gastric, duodenal, jejunal and colonic plasma leakage was determined 1 h after L-NAME.

2.4. Effect of S-nitroso-glutathione on gastrointestinal plasma leakage

In surgically manipulated rats, infusion of the NO donor *S*-nitroso-glutathione (5 μg kg^{-1} min^{-1}) into the tail vein was commenced concurrently with the administration of L-NAME (5 mg kg^{-1}, s.c.). Plasma leakage in the stomach, duodenum, jejunum and colon was measured 1 h later.

2.5. Chemicals

[^{125}I]human serum albumin was obtained from Amersham International (UK) and IZINTA (Budapest, Hungary). All the other compounds were from Sigma (Poole, Dorset, UK).

2.6. Statistics

The data are expressed as mean ± S.E.M. 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. Effect of surgical manipulation on gastrointestinal plasma leakage

Abdominal surgery and gentle manipulation of the organs provoked significant albumin leakage into the gastric and duodenal tissue (Fig. 1), and jejunal and colonic tissues (Fig. 2) over 1 h, compared with the control (resting) albumin extravasation in the stomach, duodenum, jejunum and colon of the conscious rat. The control values of plasma leakage are 51 ± 3, 118 ± 5, 136 ± 8 and 52 ± 7 μl g^{-1} tissue, respectively, over this 1 h period.

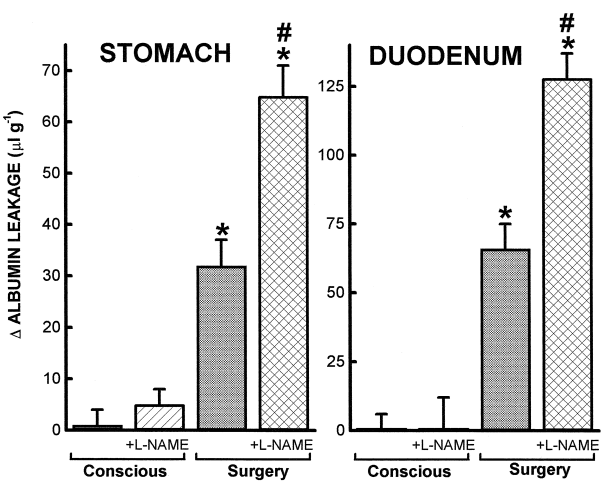

Fig. 1. Leakage of radiolabelled albumin (expressed as Δ albumin leakage, μl g^{-1} tissue) provoked by intraabdominal surgery and manipulation in the stomach and duodenum of the rat, and its aggravation by N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.). Data are shown as the mean ± S.E.M., where (n) is 8–12 for each group, and where statistical significance is given as $^*P < 0.05$ compared to conscious untreated groups.

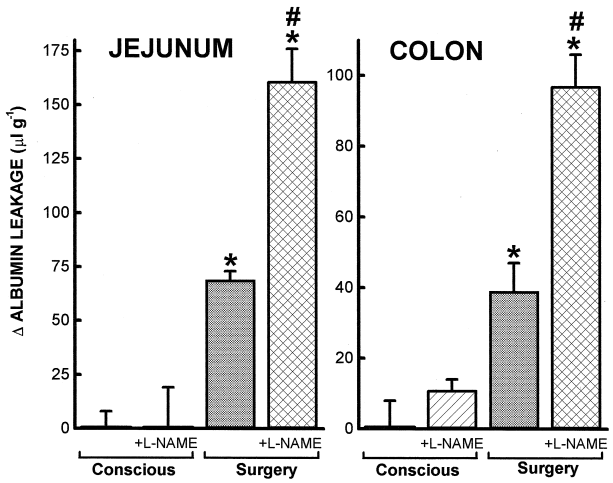

Fig. 2. Leakage of radiolabelled albumin (expressed as Δ albumin leakage, μl g^{-1} tissue) provoked by intraabdominal surgery and manipulation in the jejunum and colon of the rat, and its aggravation by N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.). Data are shown as the mean ± S.E.M., where (n) is 8–12 for each group, and where statistical significance is given as $^*P < 0.05$ compared to conscious untreated groups.

3.2. Effect of L-NAME or L-NMMA on gastrointestinal plasma leakage following surgical manipulation

In conscious unoperated control rats, administration of the NO synthase inhibitor, L-NAME (5 mg kg^{-1}, s.c.) did

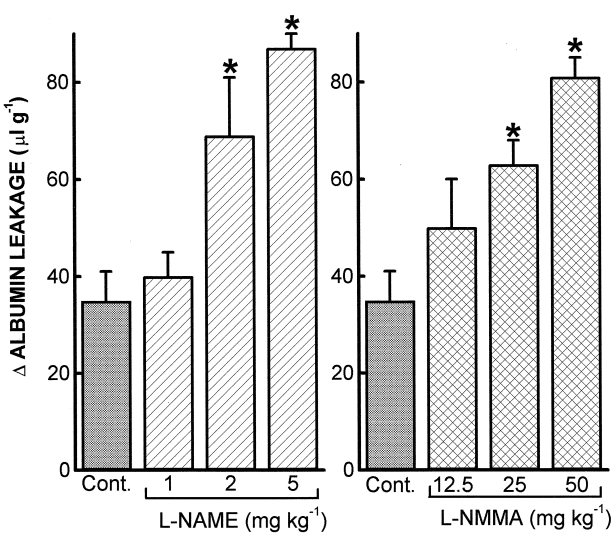

Fig. 3. Leakage of radiolabelled albumin (expressed as Δ albumin leakage, μl g^{-1} tissue) provoked by intraabdominal surgery and manipulation in the colon of the rat, and its dose-dependent aggravation by N^G-nitro-L-arginine methyl ester (L-NAME, 1–5 mg kg^{-1}, s.c.) and by N^G-monomethyl-L-arginine (L-NMMA, 12.5–50 mg kg^{-1}, s.c.). Data are shown as the mean ± S.E.M., where (n) is 6–12 for each group, and where statistical significance is given as $^*P < 0.05$ compared to the surgically manipulated control (Cont.) groups.

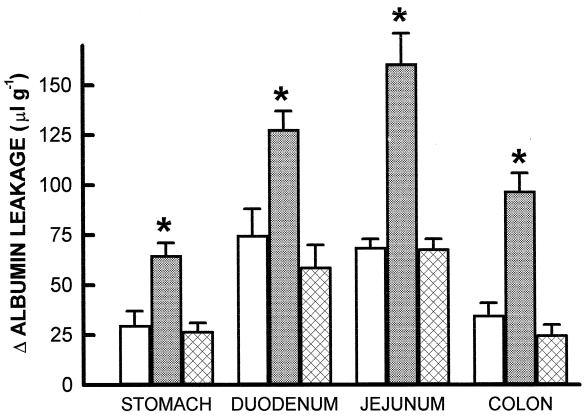

Fig. 4. Leakage of radiolabelled albumin (expressed as Δ albumin leakage, $\mu l\ g^{-1}$ tissue) provoked by intraabdominal surgery and manipulation in the stomach, duodenum, jejunum and colon of the rat (opened columns), and its aggravation by N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c., grey columns). Inhibition of albumin leakage provoked by L-NAME in the surgically manipulated rat (hatched columns) by *S*-nitroso-glutathione infusion (5 $\mu g\ kg^{-1}\ min^{-1}$, i.v.). Data are shown as the mean ± S.E.M., where (n) is 6–9 for each group, and where statistical significance is given as $^*P < 0.05$ compared to the intraabdominal surgery alone (open columns) groups.

not affect plasma leakage over 1 h in the gastric and duodenal tissue (Fig. 1), or in jejunal and colonic tissues (Fig. 2).

In surgically manipulated rats, administration of NO synthase inhibitors, L-NAME (1–5 mg kg^{-1}, s.c.) or L-NMMA (12.5–50 mg kg^{-1}, s.c.) provoked a further dose-dependent increase in plasma leakage over 1 h in the stomach and duodenum (Fig. 1), in the jejunum (Fig. 2) and in the colon (Figs. 2 and 3). This augmentation of gastrointestinal plasma leakage was inhibited near-maximally with L-arginine (300 mg kg^{-1}, s.c.) pretreatment (15 min before L-NAME) by 100 ± 12%, 90 ± 10%, 91 ± 21% and 92 ± 19% in the stomach, duodenum, jejunum and colon, respectively ($n = 4–6$, $P < 0.005$).

3.3. Effect of S-nitroso-glutathione on gastrointestinal plasma leakage

Intravenous infusion of the NO donor, *S*-nitroso-glutathione (5 $\mu g\ kg^{-1}\ min^{-1}$ for 1 h) abolished the increase in gastric, duodenal, jejunal and colonic plasma leakage induced by L-NAME (5 mg kg^{-1}, s.c.) in surgically manipulated rats, as shown in Fig. 4.

4. Discussion

In this present study, an increase in vascular permeability has been observed in gastric, duodenal, jejunal and colonic tissues in rats that had undergone intraabdominal surgery and gentle manipulation of the gastrointestinal tissues. Our study confirms previous findings in which it has shown that abdominal surgery itself provoked an elevation of vascular permeability in rats (Akerström and Lisander, 1991). The increase in albumin extravasation under the current conditions is a consequence of the gentle manipulation since it has been demonstrated that the minor surgical procedures of tracheotomy, incision of the abdominal skin or abdominal laparotomy alone did not provoke albumin leakage in the anaesthetised rat (László and Whittle, 1999). Thus, it is feasible that our model is appropriate for the investigation of the mechanisms of plasma loss during surgical procedures.

Administration of the NO synthase inhibitor, L-NAME in conscious unoperated rats did not affect basal albumin extravasation within the 1 h period, which is consistent with our previous findings (László et al., 1994a,b). However, when the NO synthase inhibitors were administered in rats with surgery and tissue manipulation, a further increase in gastrointestinal vascular permeability occurred. The actions of L-NAME could be reversed by L-arginine pretreatment. It is likely that actions of endothelial NO synthase involved, since previous studies have demonstrated that a minimum of 2 h are needed for the expression of the inducible NO synthase (Boughton-Smith et al., 1993), while in our current model, the experimental period has been only 1 h. On the basis of our present data, it appears that NO produced by endothelial NO synthase modulates the changes in vascular permeability during surgical challenge. Such events include abdominal laparotomy itself (László and Whittle, 1999) and subsequent organ manipulation. Thus, NO generated by endothelial NO synthase may play a protective role in the microcirculation against endothelial dysfunction and the consequent plasma loss during and following surgical operations.

Physiologically formed NO might protect the microcirculation by preventing the deleterious actions of neutrophils towards the vascular endothelium during surgical procedures. Polymorphonuclear leukocytes are well-known to play a crucial role in the changes in microvascular permeability during inflammatory processes leading to tissue oedema (Wedmore and Williams, 1981). Administration of L-NAME enhances the adhesion of leukocytes to the vascular endothelium, assessed by in vivo microscopy in surgically prepared animals (Kubes et al., 1991; Arndt et al., 1993). In a recent study, the increase in plasma leakage by L-NAME, in rats with abdominal laparotomy alone, was abolished by the pretreatment of a rabbit anti-rat neutrophil serum suggesting the involvement of neutrophils in these events (László and Whittle, 1999). Thus, endogenous NO formed by endothelial NO synthase may counteract to the effects of neutrophil-derived mediators which are released in response to surgical trauma.

In our present study, administration of the NO donor, *S*-nitroso-glutathione attenuated plasma extravasation provoked by L-NAME and surgical manipulation. This protection by the NO donor may involve actions on neutrophils, since NO decreases neutrophil function and their adhesion

to the vascular endothelium both in vivo and in vitro (Ma et al., 1993; Moilanen et al., 1993; Granger and Kubes, 1994).

Our present results thus suggest that the minor surgical intervention of opening the abdominal cavity and a gentle handling of the stomach or the small intestine lead to microvascular changes in these tissues. This process appears to be modulated by NO, since inhibition NO synthase substantially increased plasma extravasation from the gastrointestinal organs under these conditions. The mechanisms underlying this apparent microvascular priming are unknown, but may reflect neuronal or humoral stimulation as a consequence of the surgical stress, as well as the activation of the neutrophil. The current data suggest that should NO formation be compromised during surgical procedures, plasma and fluid loss would be augmented, events which could lead to hypovolaemia, as well as oedema formation and a decreased tissue oxygenisation. It is possible therefore that administration of NO donors during major surgical interventions may be beneficial in preventing any subsequent vascular endothelial dysfunction and the consequent plasma loss.

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