Adverse effects of pneumoperitoneum on renal function: Involvement of the endothelin and nitric oxide systems

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Abstract

Background: Increased intra-abdominal pressure (IAP) during laparoscopy adversely affects kidney function. The mechanism underlying this phenomenon is largely unknown. Objective: This study was designed to investigate the involvement of Endothelin-1 (ET-1) and nitric oxide (NO) systems in IPA-induced renal dysfunction.

Methods: Rats were subjected to IAP of 14 mmHg for 1h followed by a deflation for 60 min (recovery). Four additional groups were pretreated with: 1) ABT-627, an ETA antagonist, 2) A-192621, an ETB antagonist, 3) nitroglycerine-NTG, 4) L-NAME, NOS inhibitor before IAP. Urine flow rate (V), absolute Na+ excretion (UNaV), glomerular filtration rate (GFR), and renal plasma flow (RPF), were determined.

Results: Significant reductions in kidney function and hemodynamics were observed when IAP was applied: V decreased from 8.1±1.0 to 5.8±0.5µl/min, UNaV from 1.08±0.31 to 0.43±0.10 µEq/min (P<0.05), GFR from 1.84±0.12 to 1.05±0.06 ml/min (-46.9±2.7% from baseline) and RPF from 8.62±0.87 to 3.82±0.16 ml/min (-54±3.5% from baseline). When the animals were pretreated with either ABT-627 or A-192621 given alone or combined, the adverse effects of IAP on GFR, RPF, V and UNaV were significantly augmented. When the animals were pretreated with NTG, the adverse effects of pneumoperitoneum on GFR and RPF were substantially improved. In contrast, pretreatment with L-NAME remarkably aggravated pneumoperitoneum-induced renal dysfunction. Conclusion: Decreased renal excretory function and hypofiltration are induced by increased IAP. These effects are related to impairment of renal hemodynamics, and could be partially ameliorated by pretreatment with NTG, and aggravated by NO and ET blockade.
**Key words:** Pneumoperitoneum, Renal function, Endothelin, Nitric oxide, Rat.
Introduction

Pneumoperitoneum at pressure above 10 mmHg during laparoscopic surgery has been shown to produce transient oliguria, and reduced glomerular filtration rate (GFR) and renal blood flow (RBF) (3,9,16,33,34). Despite much research in this field, the systemic physiologic consequences of CO₂ pneumoperitoneum and the mechanisms underlying its adverse effects on renal hemodynamics and excretory function are not fully understood (9). However, there is compelling experimental evidence that the adverse renal effects induced by pneumoperitoneum are affected by the level of intra-abdominal pressure (IAP), volume status, degree of hypercarbia, positioning, and individual hemodynamic and renal reserves (9,10). Additional factors that may affect renal function during pneumoperitoneum include direct compression of the renal parenchyma and renal vein (4,17), increased resistance in the renal vasculature (47), and release of vasoconstrictors such as vasopressin, angiotensin II, catecholamines, and endothelin (ET-1) (1,13). The latter is a very potent natural mammalian vasoconstrictor agent (25), acting on the cardiovascular and renal systems and other target organs by binding to two major types of receptors, ET_A and ET_B (2,25,36). High abundance of ET_A receptors has been detected in the aorta, heart, and kidney, whereas ET_B receptors are expressed mainly in the endothelium and tubular epithelial cells of the collecting duct (24,25,26,40). Activation of ET_A receptors on vascular smooth muscle cells (VSMC) increases intracellular Ca^{2+} levels, leading to prolonged vasoconstriction and cell proliferation (31,35,41). In contrast, activation of ET_B receptors, present on endothelial cells, induces the release of nitric oxide (NO) and prostaglandins, thus provoking transient vasodilation (41,43,44). While it is accepted that ET_A and ET_B receptors mediate vasoconstriction and vasodilation, respectively, several studies have demonstrated that ET_B receptors present on VSMC can elicit vasoconstriction (6,7,8,19,20,21). Thus, differences in tissue-specific expression and density of the two receptor subtypes, the tissue concentration
of the ET-1, and the preexisting state of the vascular bed, determine the type and magnitude of vascular response (vasoconstriction or vasodilation) of this peptide (35).

The kidney is both a target organ and a major source of ET-1 production (25,35). The highest concentrations of immunoreactive ET-1 in the body have been detected in the renal medulla (23). In addition, gene expression and immunoreactive peptides of ET-1 and its receptors have been demonstrated in the renal tissue, especially in the medullary region (23,26,40,42,45). Interestingly, in vivo studies revealed that administration of ET-1 resulted in reduced renal hemodynamics and kidney dysfunction similar to those found during pneumoperitoneum (9). For instance, whole-organ studies in intact rats have demonstrated that a short-lasting infusion of ET into the renal artery decreases renal plasma flow (RPF), glomerular filtration rate (GFR) and urine volume (5,22,38,46). Similarly, a long-term infusion of ET into conscious dogs results in increased renal vascular resistance and decreased GFR and RPF (5,46). Hence, it is proposed that activation of the ET system may be involved in pneumoperitoneum-induced renal dysfunction. In line with assumption, high plasma levels of ET-1 were measured in dogs, subjected to 5 h of elevated IAP, within 20 min of insufflation (15). Likewise, an elevation in local renal ET-1 expression has recently been reported in experimental pneumoperitoneum (1). Unfortunately, these studies did not examine thoroughly the effects of selective ET receptor antagonists on renal dysfunction associated with elevated IAP.

Additional autocrine/paracrine system within the kidney that also influences the vascular and tubular function, and may interact with the actions of the ET system is nitric oxide (NO). In this regard, several studies have demonstrated that medullary NO plays a pivotal role in the regulation of renal medullary hemodynamics and excretory function (27,28,30). Previously, we (14,18) and others (48) have shown that the renal vasodilatory actions of ETB as well as its stimulatory effects on water and sodium excretion are mediated through generation of NO.
Similarly, Mattson et al (32) have demonstrated that chronic intravenous administration of the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) selectively decreases renal medullary blood flow, causes sodium and water retention and leads to hypertension. Similar manifestations were noticed in rats with collecting duct-specific knockout to the ETB receptor (12). Taken together, these findings appeal to the notion that alterations in the endogenous NO system may contribute to the altered renal hemodynamics and kidney function seen in pneumoperitoneum.

Therefore, the present study was designed to explore the effects of acute blockade of the \( \text{ET}_A \) and \( \text{ET}_B \) receptors and the involvement of the NO system on renal hemodynamics and function in rats with pneumoperitoneum at pressure of 14 mmHg.

**Materials and Methods**

Studies were conducted on male Sprague Dawley rats (Harlan Laboratories, Ltd., Jerusalem) weighing 290-320 g. The animals were fed standard rat chow containing 0.5% NaCl and tap water *ad libitum*. All experiments were performed according to the guidelines of the committee for the supervision of animal experiments, Technion, IIT. Rats were anesthetized with Inactin (100 mg/kg, i.p.), placed on a thermo-regulated (37°C) surgical table, and prepared for hemodynamic and clearance studies (11). After tracheotomy, polyethylene tubes (PE50) were inserted into the carotid artery and jugular vein, for blood pressure monitoring and infusion of various solutions, respectively. The abdomen was opened by a small midline incision and the urinary bladder was catheterized with PE50 for urine collection. A solution of 2% of inulin and 0.5% p-aminohippurate (PAH) in 0.9% saline was continuously infused at a rate of 1.0-1.5% of body weight per hour throughout the experiment. Arterial blood
pressure was continuously monitored with a pressure transducer (model 156PC05GW; Microswitch, Freepoint, IL) connected to the carotid arterial line.

**The experimental model of pneumoperitoneum:** A small incision in the lower third between the xiphoid and pubis was made, through which a regular Veress needle was inserted into the abdominal cavity. A pneumoperitoneum of 14 mmHg was established with CO2 gas supply to maintain IAP at the desired level using a special insufflator (Aesculap, Tuttlingen, Germany) connected to the Veress needle. The muscle layer and skin layer of the abdominal wall were closed separately by silk sutures in an airtight manner. This IAP pressure (14 mmHg) was chosen based on preliminary experiments where incremental increases in IAP from 0 to 7 and 14 were applied. While the latter was effective in causing renal dysfunction, IAP of 7 mmHg did not affect kidney function and hemodynamics (Data not shown). Moreover, IAP of ~14 mmHg is the pressure utilized by surgeons during laparoscopic surgery (9).

**Clearance studies:** Rats were prepared for clearance studies as described above. After a 60 minute equilibration period, two baseline clearance periods of 30 minutes each were obtained (IAP=0). The rats were then subjected to one of the following experimental protocols:

**Group A:** Seven rats were subjected to IAP of 14 mmHg over one hour, followed by deflation period of 1 hour (Recovery). Two clearance periods were obtained during baseline, vehicle/drug treatment, IAP and recovery period. Blood samples were obtained in the midst of every 2\(^{nd}\) clearance period (See below). The two collection periods during baseline, drug treatment and recovery were averaged and combined.
**Group B:** Six-seven rats were pretreated with 1) ABT-627, an antagonist of ET\textsubscript{A} receptor (1.0 mg/kg/h, i.v), and 2) A-192621, an antagonist of ET\textsubscript{B} receptor (3.0 mg/kg/h, i.v) given alone or combined beginning 60 min before the application of 14 mmHg insufflation pressure for 1 hour, followed by desufflation to 0 mm Hg (Recovery). Two clearance periods were obtained during ET antagonists infusion alone, ET antagonists +IAP of 14 mmHg, and the recovery period. Previous studies from our laboratory have demonstrated that these blockers are highly selective at the applied doses (11).

**Group C:** Seven additional rats were pretreated with a non-depressor dose of nitroglycerine-NTG (prime 1.5 µg/kg and sustained infusion of 15 µg/kg/h, i.v) beginning 60 min before the application of 14 mmHg insufflation pressure for 1 hour, followed by desufflation to 0 mm Hg (Recovery). Two clearance periods were obtained during baseline, NTG infusion alone, NTG+IAP of 14 mmHg, and the recovery period. Blood samples were obtained in the midst of every 2\textsuperscript{nd} clearance period.

**Group D:** Six additional rats were pretreated with nitro-L-arginine methyl ester L-NAME (100 mg/L) added to drinking water for 4 days before the experiment. On the fifth day, two basal clearance periods were obtained before rats were subjected to IAP of 14 mmHg over one hour, followed by a deflation period of 1 hour (Recovery). Two clearance periods were obtained during baseline, insufflation, and recovery periods. Blood samples were obtained in the midst of every 2\textsuperscript{nd} clearance period (see below).
Chemical Analysis

Urine volume was determined gravimetrically. Plasma samples were separated by centrifugation, and the concentrations of sodium in plasma and urine were determined by a flame photometer (model IL 943, Instrumentational Laboratory). Concentrations of inulin and PAH, in plasma and urine samples, were measured by the colorimetric anthrone method. Renal perfusion flow (RPF) and glomerular filtration rate (GFR) were estimated as the infusion clearance of PAH and inulin, respectively.

Statistical analysis

One-way analysis of variance (ANOVA) for repeated measures, followed by the Dunnett test, was used for comparison of treatment values with baseline in each group or with corresponding values in control group. A value of $p \leq 0.05$ was considered statistically significant. Data are presented as mean± S.E.M.

Results

1- Effects of short term increase in IAP on renal clearance parameters and renal hemodynamics: Figure 1 depicts urinary flow rate, absolute and fractional Na+ excretion and mean arterial pressure of rats that underwent incremental increases in IAP from 0 to 14 mmHg. Significant reductions in these parameters were observed when IAP of 14 mmHg was applied for 60 min.: $V$ decreased from 8.1±1.0 to 5.8±0.5µl/min ($P \leq 0.05$) (-19.6±17.1% from baseline); and $U_{Na}V$ from 1.08±0.31 to 0.43±0.10 µEq/min ($P<0.05$) (-26.0±26.7% from baseline). These alterations in excretory functions were associated with a maximal decline in GFR from 1.84±0.12 to 1.05±0.06 ml/min, $P<0.05$, (-46.9±2.7% from baseline) and RPF from 8.62±0.87 to 3.82±0.16 ml/min, $P<0.05$, (-54±3.5% from baseline) (Fig. 2), without a significant
change in MAP (from 110.2±4.6 to 107.8±6.4, P=NS) (Table 1). Both the excretory parameters and renal hemodynamics returned to baseline values following deflation period of 1 hour (Recovery) (Figures 1 & 2). The current findings indicate that IAP of 14 mmHg, significantly decreased GFR and RBF in association with impairment of urine output and sodium excretion.

2- Effects of ET<sub>A</sub> and ET<sub>B</sub> antagonists on kidney function and renal hemodynamic response to pneumoperitoneum: To further elucidate possible involvement of the ET system in the pathogenesis of pneumoperitoneum-induced renal dysfunction, studies were repeated with rats pretreated with either ABT-627 or A-192621 given alone or combined.

**ET<sub>A</sub> antagonist:** Pretreatment with ABT-627 aggravated the adverse effects of elevated IAP on V and U<sub>Na</sub>V: V decreased from 17.0±4.5 to 3.7±0.4 µl/min (P<0.05) (-68.8±6.4% from baseline), and U<sub>Na</sub>V from 0.83±0.48 to 0.08±0.04 µEq/min (P<0.05) (-73.0±9.6% from baseline) (Fig. 1). Besides its effects on renal excretory function, ABT-627 worsened the influence of pneumoperitoneum on renal perfusion: GFR decreased from 1.5±0.17 to 0.47±0.07 ml/min, P<0.05, (-67.9±4.8% from baseline) and RPF from 6.0±0.7 to 2.16±0.58 ml/min, P<0.05, (-67±4.9% from baseline) (Fig. 2). Treatment with ABT-627 produced slight decrease in MAP from 113.8±2.9 to 105.8±2.1 (P=NS) 60 min after the elevation of IAP (Table 1). It should be emphasized that the magnitude of these decreases in V, U<sub>Na</sub>V, GFR and RPF, calculated as % change from baseline was significantly greater compared to untreated rats that underwent identical insufflation (Fig. 1&2). Notably, both GFR and RBF substantially increased during the recovery period, yet V and U<sub>Na</sub>V decreased or unchanged, respectively. These results suggest that the recovery of renal
hemodynamics is faster than the recovery of the tubular function which is known to be very sensitive to hypoperfusion.

**ETβ antagonist:** Similar to ABT-627, pretreatment with A-192621 aggravated the adverse effects of pneumoperitoneum on V and UNaV: V decreased from 33.3±9.0 to 8.8±0.4 µl/min (P<0.05) (-75±6.1% from baseline), and UNaV from 1.52±0.53 to 0.35±0.02 µEq/min (P<0.05) (-77.3±4.3% from baseline) (Fig. 1). In addition, A-192621 augmented the adverse influence of pneumoperitoneum on renal perfusion: GFR decreased from 2.5±0.3 to 0.61±0.09 ml/min, P<0.05, (-76.0±7.4% from baseline) and RPF from 7.8.0±0.6 to 2.6±0.8 ml/min, P<0.05, (-82.8±4.0% from baseline) (Fig. 2). Treatment with A-192621 significantly increased MAP from 119.7±2.4 to 131.1±4.0 and 138.7±4.3 (P<0.05) 30 and 60 min after the elevation of IAP, respectively (Table 1). The magnitude of these decreases in V, UNaV, GFR and RPF, calculated as % change from baseline, was significantly greater compared with untreated rats that underwent identical insufflation (Fig. 1&2).

**ETα+ETβ antagonists:** Co-administration of both ABT-627 and A-192621 resulted in aggravation of renal excretory function and renal hemodynamics caused by elevated IAP: V decreased from 14.8. ±1.6 to 3.8±1.0 µl/min (P<0.05) (-68.8±6.4% from baseline), and UNaV from 2.44±0.48 to 0.34±0.12 µEq/min (P<0.05) (-86.3±4.3% from baseline), GFR decreased from 1.65±0.17 to 0.27±0.08 ml/min, P<0.05, (-81.0±5.6% from baseline) and RPF from 12.64±3.3 to 2.2±0.8 ml/min, P<0.05, (-86.4±5.4% from baseline) (Fig. 1&2), without significantly affecting MAP (Table 1). The magnitude of these decreases in V, UNaV, GFR and RPF, calculated as % change from baseline, was comparable to those obtained when each antagonist was administered alone (Fig. 2). Similar to rats treated with ETA antagonist, treatment with combined ETA and ETB antagonists resulted in remarkable increase in GFR (but
not RPF) during the recovery period, yet V and U_{Na}V decreased or unchanged, respectively.

3- Effects of NO donor or NO synthase inhibition on renal hemodynamic response to pneumoperitoneum

This protocol was designed to investigate the involvement of NO system in the pathogenesis of pneumoperitoneum-induced renal dysfunction. Administration of a non-depressor dose of NTG maintained urinary flow and sodium excretion during IAP of 14 mmHg (Fig. 3). However, both V and U_{Na}V still decreased relatively to the NTG-enhanced basal values: V decreased from 16.8±1.1 to 8.9±0.9 µl/min (-51.3±7.1%) (P<0.05) and U_{Na}V decreased from 1.7±0.4 to 1.14 µEq/min (-33.2±27.0%) (P<0.05), after 60 min of insufflations (Fig 3), without a significant change in MAP (107.6±2.7 to 108.4±4.2 mmHg) (Table 1).

In line with its beneficial renal excretory effects during pneumoperitoneum, NTG significantly attenuated the decrease in renal hemodynamics after 60 min, but not 30 min of insufflation. GFR decreased from 1.84±0.08 to 1.14±0.2 (-40.6±8.7%) (P<0.05), and 1.48±0.14 ml/min (-23.8±7.2%) (P<0.05), 30 and 60 min after insufflation, respectively (Fig 4). Similarly, RPF decreased from 9.59±1.0 to 4.73±0.9 (-34.9±15.4%) (P<0.05) and 6.84±0.7 ml/min (-16.7.6±11%) (P=NS), at 30 and 60 min respectively. It should be emphasized that the magnitude of the decreases in GFR and RBF during the second 30 min of insufflation, calculated as % change from baseline was significantly lower compared to untreated rats that underwent identical insufflations (Fig 4). Despite of the beneficial effects of NTG on renal hemodynamics, both GFR and RBF still dropping by 25-40% during increased IAP.
compared to baseline values. Both the excretory parameters and renal hemodynamics returned to values above normal following deflation period of 1 hour (Recovery). These results suggest that NO plays a beneficial role in maintaining renal function during pneumoperitoneum. In agreement with this notion, pretreatment with L-NAME remarkably aggravated the renal excretory function as well as hypoperfusion/hypofiltration associated with pneumoperitoneum. V decreased from 13.0±0.85 to 3.4±0.7 µl/min (-70±8.5%) (P<0.05) and U₅NaV from 0.37±0.16 to 0.12±0.08 µEq/min (-55.7±5.6%) (P<0.05), after 60 min of insufflations (Fig. 5). As expected, basal MAP was significantly higher in L-NAME treated animals compared to controls (137.0±5.0 vs. 110.2±4.6, P<0.05) (Table 1). Besides aggravating the adverse effects of pneumoperitoneum on renal excretory function, L-NAME remarkably worsened the pneumoperitoneum-induced hypoperfusion/ hypofiltration. GFR decreased from 1.70±0.15 to 0.42±0.13 (-73.8±7.7%) (P<0.05) and 0.62±0.2 ml/min (-58.8±14.0%) (P<0.05), 30 and 60 min after abdominal insufflation of L-NAME pretreated rats, respectively (Fig. 6). RPF decreased from 5.41±0.35 to 1.27±0.35 (-74.3±8.87%) (P<0.05) and 1.8±0.6 ml/min (-50.3±11.9%) (P<0.05), at 30 and 60 min, respectively (Fig. 6). Notably, the magnitude of these decreases in GFR and RBF, calculated as % change from baseline, was significantly higher in L-NAME treated animals compared with untreated rats that underwent identical insufflations (Fig. 6).

**Discussion**

The findings of the present study provide novel information on the effects of pneumoperitoneum on kidney function and renal perfusion and the mechanisms underlying the adverse physiological consequences of this surgical procedure.
As expected, we demonstrated that rats subjected to 14 mmHg of pneumoperitoneum show reductions in both renal hemodynamics and excretory function. Selective ET<sub>A</sub> or ET<sub>B</sub> blockers given separately or combined, aggravated these effects. Similarly, NO inhibition enhanced the pneumoperitoneum-induced renal dysfunction, whereas pretreatment with non-depressor doses of NTG significantly ameliorated the adverse effects of IAP on GFR and RPF. Although, NTG maintained urinary flow and sodium excretion during IAP of 14 mmHg, both V and U<sub>Na</sub>V still decreased relatively to the NTG-enhanced basal values. These findings suggest that both ET and NO systems play an important beneficial role in maintaining renal hemodynamic and kidney function during pneumoperitoneum.

A role of ET-1 in the pathogenesis of the adverse renal effects of pneumoperitoneum has been suggested, based on the following findings: first, Hamilton and his colleagues (15) reported that plasma levels of ET-1 are elevated in dogs, subjected to 5 h of elevated IAP, within 20 min of insufflation. Moreover, Ambrose and his colleagues (1) demonstrated increased expression of ET-1 gene in the renal vasculature and proximal tubule of rats subjected to 30 min of tissue pneumoperitoneum. In addition, infusion of ET-1 into the kidney produced decreases in RBF, GFR and V, similar to those found during pneumoperitoneum (25). Collectively, this suggests that upregulation of ET system could play a part in the renal dysfunction associated with pneumoperitoneum and that blockade of this system might improve kidney function. In line with this notion; preliminary data in a rat pneumoperitoneum model indicate that administration of Ro 61-0612, a non-selective ET<sub>A</sub> and ET<sub>B</sub> receptor antagonist, before and during insufflation improved kidney function and renal hemodynamics during pneumoperitoneum (1,39). To the best of our knowledge, the current study is the first one that examines whether selective ET<sub>A</sub>
or ET<sub>B</sub> blockade can alter the changes in kidney function and renal hemodynamics caused by pneumoperitoneum. In line with its physiological vasodilatory action (14, 19,25), blockade of ET<sub>B</sub> receptor aggravated the fall in GFR and RPF during pneumoperitoneum. This observation is further supported by our previous findings that ET<sub>B</sub> receptor is preferably expressed in the outer and inner medulla, mainly in the vasa recta, thick ascending limb of Henles' loop and collecting duct (11). Moreover, our finding that ET<sub>B</sub> blockade worsened the oliguria characterizing pneumoperitoneum, supports the role of tubular ET<sub>B</sub> receptors in mediating the diuretic effect of ET-1 under physiological conditions (11,18,25).

Unexpectedly, ETA blockade did not prevent the decline in V, U<sub>Na</sub>V, GFR, and RPF induced by pneumoperitoneum, rather it caused significant decreases in these parameters comparable to those obtained with ET<sub>B</sub> antagonist. Taking into account the diuretic action of ET<sub>B</sub> receptor, these effects are unexpected and could not be explained by changes in blood pressure, since MAP declined slightly and insignificantly following the administration of ET<sub>A</sub> blocker. Moreover, one may expect that blockade of ET<sub>A</sub>, which predominantly localized to the peritubular capillaries and mediates vasoconstriction, should result in increase in RPF and GFR. Although the bulk of evidence supports a vasodilatory function of ET<sub>B</sub> receptors, few studies revealed that in some vascular beds, ET-1 elicits constriction through both receptors subtypes (8,19,20). Activation of ET<sub>B</sub> receptor with selective agonists such as sarafotoxin 6c produced transient depressor response followed by a longer pressor phase (7). Similarly, BQ3020, an ETB agonist, is potent constrictor of renal and mesenteric vasculature beds (21). Based on these contradictory results, it is widely believed that the endothelial ET<sub>B</sub> receptor mediates dilatation and the smooth muscle cell ET<sub>B</sub> receptor mediates constriction (6,19,37).
Dual blockade of ET\textsubscript{A} and ET\textsubscript{B} resulted in aggravation of the renal excretory function and renal hypoperfusion caused by elevated IAP. However, the magnitude of these decreases in V, U\textsubscript{NaV}, GFR and RPF, was only slightly higher than those obtained when each antagonist was administered alone, suggesting that blockade of the ET system either at ET\textsubscript{A} or ET\textsubscript{B} level is sufficient to eliminate the beneficial effects of this system during pneumoperitoneum. These results are at odds with those reported that administration of Ro 61-0612, a mixed ET\textsubscript{A} and ET\textsubscript{B} blocker, attenuated the fall in GFR and oliguria during pneumoperitoneum (1,39). It should emphasized that these authors applied similar IAP and drug infusion protocol, however the pneumoperitoneum was established for only 15 minutes, which most likely produce less profound insult to renal function as compared with the 60 min applied in the present study.

Because endothelium-derived NO is important in the regulation of RBF and kidney function (28,29), we tested the hypothesis whether altered activity of the NO system may be involved in the pathogenesis of the reduced RBF and kidney function characterizing pneumoperitoneum. Indeed, the most prominent finding in the present study was the effects of NO modulations on the adverse renal effects of elevated IAP. In that respect, rats that received NTG displayed attenuated renal vasoconstrictive response to pneumoperitoneum, whereas rats pretreated with L-NAME displayed a higher sensitivity to this surgical procedure, suggesting a greater dependence of the kidney on NO as a beneficial counter-regulatory system during pneumoperitoneum. This conclusion is compatible with the notion that NO plays a prominent role in the control of renal hemodynamics and tubular function (28,29). NO is constitutively produced from its precursor, L-arginine, by the enzyme eNOS and acts on adjacent smooth muscle cells to exert vasodilatory tone on the renal microvasculature,
primarily the afferent arteriole. In addition, NO affects renal function by modulating
tubuloglomerular feedback and renin release and by altering tubular salt reabsorption
(28,29). These actions are thought to be mediated by NO generated in vascular
endothelial cells, mesangial cells, macula densa, and epithelial tubular cells.

**Perspective and significance**

Our study demonstrates for the first time the involvement of the NO system in the
adaptive changes in renal hemodynamics and kidney function during the induction of
pneumoperitoneum. While blockade of ET or NO systems aggravates the
pneumoperitoneum-induced renal hypoperfusion and oliguria, pretreatment with NTG
substantially attenuates the adverse effects of elevated IAP on kidney function and
renal hemodynamics. Our data may have potential therapeutic implications and
suggest that pretreatment with NO donor may be beneficial in the setting of
laparoscopic surgery. Future studies are requested to evaluate the effects of
pneumoperitoneum on the expression of the various NOS isoforms and production of
NO in the renal tissue.
References


Table 1: Mean arterial blood pressure in the different experimental groups

<table>
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<tr>
<th>Group</th>
<th>Baseline</th>
<th>14 mmHg (30 min)</th>
<th>14 mmHg (60 min)</th>
<th>Recovery</th>
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<td>IAP+Vehicle</td>
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*, P<0.05 vs baseline, #, P<0.05 vs IAP+Vehicle group.
**Figure legends:**

**Figure 1:** Effects of 14 mmHg insufflations with CO2 on: A) Urinary flow (V) and C) Urinary sodium excretion (U\textsubscript{Na}V) in the presence and absence of ABT-627, an ET\textsubscript{A} antagonist (1.0 mg/kg/h, i.v), or A-192621, an ET\textsubscript{B} antagonist (3.0 mg/kg/h, i.v) (n=6-7). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#) P<0.05 vs. untreated pneumoperitoneum. Baseline data represent the 1h vehicle/antagonist collection period.

**Figure 2:** Effects of 14 mmHg insufflations with CO2 on: A) glomerular filtration rate (GFR) and B) Renal plasma flow (RPF) in the presence and absence of ABT-627, an ET\textsubscript{A} antagonist (1.0 mg/kg/h, i.v), or A-192621, an ET\textsubscript{B} antagonist (3.0 mg/kg/h, i.v) (n=6-7). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#) P<0.05 vs. untreated pneumoperitoneum. Baseline data represent the 1h vehicle/antagonist collection period.

**Figure 3:** Effects of pretreatment with nitroglycerine (15 µg/kg/h, i.v) on pneumoperitoneum (IAP=14 mmHg)-induced renal excretory dysfunction. A) Urinary flow (V) C) Urinary sodium excretion (U\textsubscript{Na}V) (n=7). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#) P<0.05 vs. untreated pneumoperitoneum. Baseline data represent the 1h vehicle/NTG collection period.

**Figure 4:** Effects of pretreatment with nitroglycerine (15 µg/kg/h, i.v) on pneumoperitoneum (IAP=14 mmHg)-induced renal hemodynamic changes. A) Glomerular filtration rate (GFR) and C) Renal plasma flow (RPF) (n=7). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#)
P<0.05 vs. untreated pneumoperitoneum. Baseline data represent the 1h vehicle/NTG collection period.

**Figure 5:** Effects of pretreatment with L-NAME (100 mg/L in drinking water) on pneumoperitoneum (IAP=14 mmHg)-induced renal excretory dysfunction. A) Urinary flow (V) C) Urinary sodium excretion (U\textsubscript{Na}V) (n=6). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#) P<0.05 vs. untreated pneumoperitoneum.

**Figure 6:** Effects of pretreatment with L-NAME (100 mg/L in drinking water) on pneumoperitoneum (IAP=14 mmHg)-induced renal hemodynamic changes. A) Glomerular filtration rate (GFR) and C) Renal plasma flow (RPF) (n=6). Untreated animals served as controls (n=7). Data are expressed as absolute values (A and C) and as % change from baseline values (B and D). (*) P<0.05 vs. Baseline, (#) P<0.05 vs. untreated pneumoperitoneum.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6