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

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1Department of Physiology and Biophysics, Ruth & Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, and 2Department of Vascular Surgery and Kidney Transplantation, 3Department of General Surgery “A”, and 4Department of Anesthesiology, Rambam Human Health Care Campus, Haifa, Israel

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Abassi Z, Bishara B, Karram T, Khatib S, Winaver J, Hoffman A. Adverse effects of pneumoperitoneum on renal function: involvement of the endothelin and nitric oxide systems. Am J Physiol Regul Integr Comp Physiol 294: R842–R850, 2008.—Increased intra-abdominal pressure (IAP) during laparoscopy adversely affects kidney function. The mechanism underlying this phenomenon is largely unknown. This study was designed to investigate the involvement of endothelin (ET)-1 and nitric oxide (NO) systems in IAP-induced renal dysfunction. Rats were subjected to IAP of 14 mmHg for 1 h, 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; and 4) Nω-nitro-L-arginine methyl ester, a NO synthase inhibitor, before IAP. Urine flow rate (V), absolute Na+ excretion (UNaV), glomerular filtration rate (GFR), and renal plasma flow (RPF) were determined. Significant reductions in kidney function and hemodynamics were observed when IAP was applied. V decreased from 8.1 ± 1.0 to 5.8 ± 0.5 μl/min, UNaV from 1.08 ± 0.31 to 0.43 ± 0.10 μeq/min, 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 nitroglycerine, the adverse effects of pneumoperitoneum on GFR and RPF were substantially improved. In contrast, pretreatment with Nω-nitro-L-arginine methyl ester remarkably aggravated pneumoperitoneum-induced renal dysfunction. In 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 nitroglycerine and aggravated by NO and ET blockade.

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 physiological 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, ETα and ETβ (2, 25, 36). High abundance of ETα receptors has been detected in the aorta, heart, and kidney, whereas ETβ receptors are expressed mainly in the endothelium and tubular epithelial cells of the collecting duct (24, 25, 26, 40). Activation of ETα receptors on vascular smooth muscle cells (VSMC) increases intracellular Ca2+ levels, leading to prolonged vasoconstriction and cell proliferation (31, 35, 41). In contrast, activation of ETβ 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 ETα and ETβ receptors mediate vasoconstriction and vasodilation, respectively, several studies have demonstrated that ETβ receptors present on VSMC can elicit vasoconstriction (6–8, 19–21). 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 active 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 shortlasting infusion of ET-1 into the renal artery decreases renal plasma flow (RPF), GFR, and urine volume (5, 22, 38, 46). Similarly, a long-term infusion of ET-1 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 this assumption, high plasma levels of ET-1 were measured in...
dogs, subjected to 5 h of elevated IAP, within 20 min of insufflation (15). Similarly, 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.

An 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 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, our laboratory (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 N\textsuperscript{G}-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 ET\textsubscript{B} 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 ET\textsubscript{A} and ET\textsubscript{B} receptors and the involvement of the NO system on renal hemodynamics and function in rats with pneumoperitoneum at a pressure of 14 mmHg.

MATERIALS AND METHODS

Studies were conducted on male Sprague-Dawley rats (Harlan Laboratories, Jerusalem, Israel) weighing 290–320 g. The animals were fed standard rat chow containing 0.5% NaCl and tap water ad libitum. All experiments were approved by, and performed according to the guidelines of, the committee for the supervision of animal experiments, Technion, IIT.

Rats were anesthetized with Inactin (100 mg/kg ip), placed on a thermoregulated (37°C) surgical table, and prepared for hemodynamic and clearance studies (11). After tracheotomy, polyethylene tubes (PE\textsubscript{50}) 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 PE-50 for urine collection. A solution of 2% of inulin and 0.5% p-aminomhippurate (PAH) in 0.9% saline was continuously infused at a rate of 1.0–1.5% of body weight per hour throughout the experiment. Mean arterial blood pressure (MAP) was continuously monitored with a pressure transducer (model 156PC05GWL; Microswitch, Freepoint, IL) connected to the carotid arterial line.

**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 CO\textsubscript{2} gas supply to maintain IAP at the desired level using a special insufflator (Aesculap, Tuttinglen, 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 in which incremental increases in IAP from 0 to 7 and 14 mmHg 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-min equilibration period, two baseline clearance periods of 30 min 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 1 h, followed by deflation period of 1 h (recovery). Two clearance periods were obtained during baseline, vehicle/drug treatment, IAP, and recovery period. Blood samples were obtained in the midst of every second clearance period (Fig. 1). The two collection periods during baseline, drug treatment, and recovery were averaged and combined.

*Group B.* Six or seven rats were pretreated with 1) ABT-627, an antagonist of ET\textsubscript{A} receptor (1.0 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} iv), and 2) A-192621, an antagonist of ET\textsubscript{B} receptor (3.0 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} iv), given alone or combined, beginning 60 min before the application of 14-mmHg insufflation pressure for 1 h, followed by desufflation to 0 mmHg (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 nondepressor dose of nitroglycerine (NTG) (prime 1.5 \mu g/kg and sustained infusion of 15 \mu g·kg\textsuperscript{-1}·h\textsuperscript{-1} iv), beginning 60 min before the application of 14-mmHg insufflation pressure for 1 h, followed by desufflation to 0 mmHg (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 second clearance period.

*Group D.* Six additional rats were pretreated with 1-NAME (100 mg/l) added to drinking water for 4 days before the experiment. On the 5th day, two basal clearance periods were obtained before rats were subjected to IAP of 14 mmHg over 1 h, followed by a deflation period of 1 h (recovery). Two clearance periods were obtained during baseline, insufflation, and recovery periods. Blood samples were obtained in the midst of every second clearance period (Fig. 2).
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. RPF and GFR were estimated as the clearance of the infused PAH and inulin, respectively.

Statistical Analysis

One-way 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 the control group. A value of $P < 0.05$ was considered statistically significant. Data are presented as means $\pm$ SE.

RESULTS

Effects of Short-term Increase in IAP on Renal Clearance Parameters and Renal Hemodynamics

Figure 3 depicts urinary flow rate (V), and absolute and fractional Na\(^+\) excretion (UNa\(^+\)), 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 $8.1 \pm 1.0$ to $5.8 \pm 0.5$ μl/min ($P \leq 0.05$; $-19.6 \pm 17.1\%$ from baseline), and UNa\(^+\) from $1.08 \pm 0.31$ to $0.43 \pm 0.10$ μeq/min ($P < 0.05$; $-26.0 \pm 26.7\%$ from baseline). These alterations in excretory functions were associated with a maximal decline in GFR from $1.84 \pm 0.12$ to $1.05 \pm 0.06$ ml/min ($P < 0.05$; $-46.9 \pm 2.7\%$ from baseline), and RPF from $8.62 \pm 0.87$ to $3.82 \pm 0.16$ ml/min ($P < 0.05$; $-54 \pm 3.5\%$ from baseline) (Fig. 4), without a significant change in MAP [from $110.2 \pm 4.6$ to $107.8 \pm 6.4$ mmHg; $P = $ not significant (NS)] (Table 1). Both the excretory parameters and renal hemodynamics returned to baseline values following a deflation period of 1 h (recovery) (Figs. 3 and 4). The current findings indicate that IAP of 14 mmHg significantly decreased GFR and RBF, in association with impairment of urine output and UNa\(^+\).

Effects of ET\(\alpha\) and ET\(\beta\) 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\(\alpha\) antagonist. Pretreatment with ABT-627 aggravated the adverse effects of elevated IAP on V and UNa\(^+\). V decreased from $8.1 \pm 1.0$ to $5.8 \pm 0.5$ μl/min ($P \leq 0.05$; $-19.6 \pm 17.1\%$ from baseline), and UNa\(^+\) from $1.08 \pm 0.31$ to $0.43 \pm 0.10$ μeq/min ($P < 0.05$; $-26.0 \pm 26.7\%$ from baseline). These alterations in excretory functions were associated with a maximal decline in GFR from $1.84 \pm 0.12$ to $1.05 \pm 0.06$ ml/min ($P < 0.05$; $-46.9 \pm 2.7\%$ from baseline), and RPF from $8.62 \pm 0.87$ to $3.82 \pm 0.16$ ml/min ($P < 0.05$; $-54 \pm 3.5\%$ from baseline) (Fig. 4), without a significant change in MAP [from $110.2 \pm 4.6$ to $107.8 \pm 6.4$ mmHg; $P = $ not significant (NS)] (Table 1). Both the excretory parameters and renal hemodynamics returned to baseline values following a deflation period of 1 h (recovery) (Figs. 3 and 4). The current findings indicate that IAP of 14 mmHg significantly decreased GFR and RBF, in association with impairment of urine output and UNa\(^+\).
from 17.0 ± 4.5 to 3.7 ± 0.4 μL/min (P < 0.05; -68.8 ± 6.4% from baseline), and UNaV from 0.83 ± 0.48 to 0.08 ± 0.04 μeq/min (P < 0.05; -73.0 ± 9.6% from baseline) (Fig. 3). 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. 4). Treatment with ABT-627 produced a slight decrease in MAP from 113.8 ± 9.0 to 8.8 ± 3.8 mlHg (P = NS) 60 min after the elevation of IAP (Table 1). It should be emphasized that the magnitude of these decreases in V, UNaV, GFR, and RPF, calculated as percent change from baseline, was significantly greater compared with that of untreated rats that underwent identical insufflation (Figs. 3 and 4). Notably, both GFR and RPF substantially increased during the recovery period, yet V and UNaV were 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.

*ET<sub>B</sub> 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 ± 4.0 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. 3). 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 ± 4.7% from baseline), and RPF from 7.8 ± 0.6 to 2.6 ± 0.8 ml/min (P < 0.05; -82.8 ± 4.0% from baseline) (Fig. 4). Treatment with A-192621 significantly increased MAP from 119.7 ± 3.2 to 138.7 ± 4.3 mlHg (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 percent change from baseline, was significantly greater compared with that of untreated rats that underwent identical insufflation (Figs. 3 and 4).

**ET<sub>B</sub> + ET<sub>A</sub> antagonists.** Coadministration 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)

### Table 1. Mean arterial blood pressure in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>14 mmHg (30 min)</th>
<th>14 mmHg (60 min)</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td>IAP + vehicle</td>
<td>110.2 ± 4.6</td>
<td>105.8 ± 7.0</td>
<td>107.8 ± 6.4</td>
<td>101 ± 8.3</td>
</tr>
<tr>
<td>IAP + NTG</td>
<td>107.6 ± 2.7</td>
<td>109.3 ± 4.5</td>
<td>108.4 ± 4.2</td>
<td>107.1 ± 2.7</td>
</tr>
<tr>
<td>IAP + L-NAME</td>
<td>137.0 ± 5.0&lt;sup&gt;†&lt;/sup&gt;</td>
<td>120.6 ± 8.6</td>
<td>112.2 ± 15.0</td>
<td>118.8 ± 7.5</td>
</tr>
<tr>
<td>IAP + ETA antagonist</td>
<td>113.8 ± 2.9</td>
<td>110.8 ± 3.0</td>
<td>105.8 ± 2.1</td>
<td>105.2 ± 4.0</td>
</tr>
<tr>
<td>IAP + ETB antagonist</td>
<td>119.7 ± 2.4</td>
<td>131.1 ± 4.0&lt;sup&gt;†&lt;/sup&gt;</td>
<td>138.7 ± 4.3&lt;sup&gt;†&lt;/sup&gt;</td>
<td>140.0 ± 4.62&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAP + ETA + ETB antagonists</td>
<td>120.7 ± 3.6</td>
<td>111.8 ± 5.9</td>
<td>120.0 ± 4.4</td>
<td>116.2 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg. IAP, intra-abdominal pressure; NTG, nitroglycerine; L-NAME, Nω-nitro-L-arginine methyl ester; ET, endothelin. *P < 0.05 vs. baseline. †P < 0.05 vs. IAP + vehicle group.
(Figs. 3 and 4), without significantly affecting MAP (Table 1). The magnitude of these decreases in V, UNaV, GFR, and RPF, calculated as percent change from baseline, was comparable to those obtained when each antagonist was administered alone (Fig. 4). Similar to rats treated with ET\textsubscript{A} antagonist, treatment with combined ET\textsubscript{A} and ET\textsubscript{B} antagonists resulted in remarkable increase in GFR (but not RPF) during the recovery period, yet V and UNaV were decreased or unchanged, respectively.

**Effects of NO Donor or NO Synthase Inhibition on Renal Hemodynamic Response to Pneumoperitoneum**

This protocol was designed to investigate the involvement of the NO system in the pathogenesis of pneumoperitoneum-induced renal dysfunction.

Administration of a nondepressor dose of NTG maintained V and UNaV during IAP of 14 mmHg (Fig. 5). However, both V and UNaV still decreased relative to the NTG-enhanced basal values. V decreased from 16.8 ± 1.1 to 8.9 ± 0.9 ml/min (−51.3 ± 7.1%; P < 0.05), and UNaV decreased from 1.7 ± 0.4 to 1.14 μeq/min (−33.2 ± 27.0%; P < 0.05), after 60 min of insufflations (Fig. 5), 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 ml/min (−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. 6). Similarly, RPF decreased from 9.59 ± 1.0 to 4.73 ± 0.9 ml/min (−34.9 ± 15.4%; P < 0.05) and 6.84 ± 0.7 ml/min (−16.7 ± 11%; P = NS), at 30 and 60 min, respectively. It should be emphasized that the magnitude of the decreases in GFR and RPF during the second 30 min of insufflation, calculated as percent change from baseline, was significantly lower compared with untreated rats that underwent identical insufflations (Fig. 6). Despite the beneficial effects of NTG on renal hemodynamics, both GFR and RBF still dropped by 25–40% during increased IAP compared with baseline values. Both the excretory parameters and renal hemodynamics returned to values above normal following a deflation period of 1 h (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 hyperperfusion and 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 UNaV 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. 7). As expected, basal MAP was significantly higher in l-NAME-treated animals compared with controls (137.0 ± 5.0 vs. 110.2 ± 4.6 mmHg; P < 0.05) (Table 1). Besides aggravating the adverse effects of pneumoperitoneum on renal excretory function, l-NAME remarkably worsened the pneumoperitoneum-induced hyperperfusion/hypofiltration. GFR decreased from 1.70 ± 0.15 to 0.42 ± 0.13 ml/min (−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, respectively, after abdominal insufflation of l-NAME-pretreated rats (Fig. 8). RPF decreased from 5.41 ± 0.35 to 1.27 ± 0.35 ml/min (−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. 8). Notably, the magnitude of these decreases in GFR and RPF, calculated as percent change from baseline, was significantly higher in l-NAME-treated animals compared with untreated rats that underwent identical insufflations (Fig. 8).

**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 ETA or ETB blockers, given separately or combined, aggravated these effects. Similarly, NO inhibition enhanced the pneumoperitoneum-induced renal dysfunction, whereas pretreatment with nondepressor doses of NTG significantly ameliorated the adverse effects of IAP on GFR and RPF. Although NTG maintained V and UNaV during IAP of 14 mmHg, both V and UNaV still decreased relative to the NTG-enhanced basal values. These effects. Similarly, NO inhibition enhanced the pneumoperitoneum-induced renal dysfunction, whereas pretreatment with nondepressor doses of NTG significantly ameliorated the adverse effects of IAP on GFR and RPF. Although NTG maintained V and UNaV during IAP of 14 mmHg, both V and UNaV still decreased relative 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 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 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 the 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 nonselective ETA- and ETB-receptor antagonist, before and during insufflation, improved kidney function and renal hemodynamics during pneumoperitoneum (1, 39). To the best of our knowledge, the present study is the first one that examines whether selective ETA or ETB 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 ETB receptor aggravated the fall in GFR during pneumoperitoneum (25). Collectively, this suggests that upregulation of the ET system could play a part in the renal dysfunction associated with pneumoperitoneum. In line with this notion, preliminary data in a rat pneumoperitoneum model indicate that administration of Ro 61–0612, a nonselective ETA- and ETB-receptor antagonist, before and during insufflation, improved kidney function and renal hemodynamics during pneumoperitoneum (1, 39). To the best of our knowledge, the present study is the first one that examines whether selective ETA or ETB 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 ETB receptor aggravated the fall in GFR and RPF during pneumoperitoneum. This observation is further supported by our laboratory’s previous findings that ETB 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 ETB blockade worsened the oliguria characterizing pneumoperitoneum supports the role of tubular ETB 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, UNaV, GFR, and RPF induced by pneumoperitoneum, rather it caused significant decreases in these parameters, comparable to those obtained with ETB antagonist. Taking into account the diuretic action of the ETB 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 ETA blocker. Moreover, one may expect that blockade of ETA, which predominantly localized to the peritubular capillaries and mediates vasoconstriction, should result in an increase in RPF and GFR. Although the bulk of evidence supports a vasodilatory function of ETB receptors, few studies revealed that, in some vascular beds, ET-1 elicits constriction through both receptor subtypes (8, 19, 20). Activation of ETB 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 a potent constrictor of renal and mesenteric vasculature beds (21). Based on these contradictory results, it is widely believed that the endothelial ETB receptor mediates dilatation and the smooth muscle cell ETB receptor mediates constriction (6, 19, 37).

Dual blockade of ETA and ETB resulted in aggravation of the renal excretory function and renal hypoperfusion caused by elevated IAP. However, the magnitude of these decreases in V, UNaV, 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 the ETA or ETB 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 ETA and ETB blocker, attenuated the fall in GFR and oliguria during pneumoperitoneum (1, 39). It should be emphasized that these authors applied similar IAP and drug infusion protocol; however, the pneumoperitoneum was established for
only 15 min, which most likely produced less profound insult to renal function 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 of 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 vasoconstrictor 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 counterregulatory system during a surgical procedure, indicating a greater dependence of the kidney on NO as a beneficial counterregulatory 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 endothelial NO synthase 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 renal 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 NO synthase isoforms and production of NO in the renal tissue.

**REFERENCES**


