The role of nitric oxide in the development of neurogenic pulmonary edema in spinal cord-injured rats: the effect of preventive interventions

Jiří Šedý, Josef Zicha, Jaroslav Kuneš, Aleš Hejčl, and Eva Syková

Institute of Experimental Medicine and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; Institute of Dental Research, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic; Center for Cardiovascular Research, Prague, Czech Republic; and Center for Cell Therapy and Tissue Repair and Department of Neuroscience, Second Faculty of Medicine, Charles University, Prague, Czech Republic

Submitted 11 May 2009; accepted in final form 6 August 2009

NEUROGENIC PULMONARY EDEMA (NPE) is an acute life-threatening complication following spinal cord or brain injury (4). It is characterized by marked pulmonary vascular congestion, extravasation of protein-rich edema fluid, and intra-alveolar hemorrhage (7–10). Many pathophysiological mechanisms have been implicated in the development of NPE, but the exact cascade leading to its development is still unclear (9, 10). Both the release of vasoactive substances and a severe transient sympathetic discharge are thought to participate in this process (23, 24). These processes lead to an increase in pulmonary capillary hydrostatic pressure, damage to the alveolar wall, and the leakage of fluid into the intra-alveolar space (4).

Our previous experiments showed that severe NPE could be experimentally induced by rapid epidural balloon compression of the thoracic spinal cord in rats anesthetized by 1.5% isoflu-
the Czech Republic, Prague, Czech Republic. All efforts have been made to decrease the number of animals used in the study.

**Design of the study.** Animals were randomly divided into 14 experimental groups (Table 1) according to the following criteria: 1) presence of spinal cord injury, 2) dose of isoflurane anesthesia, 3) acute or chronic L-NAME administration, and 4) pentolinium or atropine administration.

Animals were anesthetized with either 1.5% or 3% isoflurane in air (flow 300 ml/min), and an arterial catheter was inserted for the monitoring of BP and HR, and a venous catheter was inserted into the common carotid artery and internal jugular vein for the administration of L-NAME, pentolinium, or atropine; both catheters were exteriorized in the interscapular region. The animal was put in a prone position, and the balloon compression of spinal cord was performed. Rats were killed 10 min after lesioning (i.e., after balloon deflation), and the grade of NPE was independently evaluated using macroscopic visual examination of subpleural bleeding and the P index (lung weight/body weight \( \times 100 \)). Controls were healthy noninjured animals that were killed immediately after the induction of anesthesia. The possible role of isoflurane in inducing NPE per se was excluded in our previous study (18).

**Balloon compression spinal cord lesion.** To induce a spinal cord injury, we used the model of an epidural balloon compression lesion (25), as described previously (18). Briefly, under sterile conditions, a 2-cm midline incision was made at the Th10-Th1 level. The dorsal muscles were shifted laterally, and the Th10 and Th11 spinous processes were removed. A hole was drilled into the Th10 lamina with a dental drill. Then, a 2F French Fogarty catheter (Baxter Healthcare, Irvine, CA), which was filled with distilled water and connected to a 50-\( \mu \)l Hamilton syringe, was inserted into the dorsal epidural space 10 mm rostrally, to reach the Th8-Th9 spinal level. Using a micromanipulator, the balloon was rapidly inflated to 15 \( \mu \)l, and the inflated balloon was left in place for 5 min. Subsequently, the balloon was deflated and removed.

The immediate inflation of the balloon in the epidural space of the thoracic spinal channel with 15 \( \mu \)l water under 1.5% isoflurane anesthesia reliably and reproducibly produces severe neurogenic pulmonary edema (18, 19). On the other hand, animals that undergo the same procedure under 3% isoflurane anesthesia do not develop neurogenic pulmonary edema (18, 19). Therefore, in the present experiments, we used these two contrasting models, in which the role of NO, and NPE was independently evaluated using macroscopic visual examination of subpleural bleeding and the P index (lung weight/body weight \( \times 100 \)). Controls were healthy noninjured animals that were killed immediately after the induction of anesthesia.

**Evaluation of NPE.** The lungs were immediately removed from killed animal and weighed by an independent investigator. To estimate the liquid accumulation in the lungs, both lungs were weighed, and the relative pulmonary weight was calculated as the pulmonary index (lung weight/body weight \( \times 100 \)), which has been previously considered to be very sensitive to the degree of pulmonary edema (9, 10, 14, 15, 18). In all cases, a mild hematoma, maximally 1 mm in diameter, was found in the hilus area as a result of the manipulation of the pulmonary vessels during lung removal (not taken into further account). The level of pulmonary subpleural bleeding was evaluated macroscopically as “Absent” (no bleeding on the lung surface), “Grade I” (small bleeding areas, occupying not more than 10% of the lung surface), “Grade II” (medium-sized bleeding areas, occupying 11–50% of the lung surface), and “Grade III” (massive bleeding areas, occupying more than 50% of the lung surface), as described previously (18, 19). Each lung was evaluated separately. In our previous experiments, we documented that the pulmonary index and the extent of subpleural bleeding clearly correspond to the histological picture of the severity of lung edema (18, 19, 22); thus, the histology was not performed in the current experiments.

**Measurement of BP and HR changes.** Mean arterial pressure (MAP, mmHg) and HR (beats per minute [bpm]) were monitored for 5 min before the procedure, throughout the entire procedure, and for 15 min after the balloon compression of the spinal cord in groups 1–13 of this study using a PowerLab system (ADInstruments, Colorado Springs, CO).
Maximum value after balloon insertion, before spinal cord injury. The hemodynamic values were obtained at induced HR decrease, atropine (4 mg/kg iv; Sigma) was given 2 min before balloon inflation. To limit the baroreflex-system, we administered the ganglionic blocker pentolinium (5 mg/kg "chronic"). To eliminate the influence of the sympathetic nervous groups 3

recovery vs. corresponding peak values.

found after pentolinium or atropine administration from the corresponding peak values.

administration vs. corresponding control (groups 1–3).

atropine atropine

Mean arterial pressure, mmHg

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Pharmacological intervention</td>
<td>acute l-NAME</td>
<td>chronic l-NAME</td>
<td>atropine chronic l-NAME</td>
<td>atropine acute l-NAME</td>
<td>atropine chronic l-NAME</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline values</td>
<td>93±5</td>
<td>97±5</td>
<td>123±3*</td>
<td>97±1</td>
<td>105±4</td>
<td>140±6†</td>
<td>103±3</td>
<td>102±2</td>
<td>140±5§</td>
</tr>
<tr>
<td>l-NAME</td>
<td>—</td>
<td>117±5</td>
<td>—</td>
<td>122±6</td>
<td>—</td>
<td>—</td>
<td>116±3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Balloon insertion</td>
<td>99±10</td>
<td>125±7*</td>
<td>128±4†</td>
<td>114±8</td>
<td>131±5</td>
<td>154±10†</td>
<td>106±6</td>
<td>125±4</td>
<td>151±4§</td>
</tr>
<tr>
<td>Pentolinium injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>55±2</td>
<td>62±3</td>
<td>62±4</td>
<td>77±3</td>
<td>123±1</td>
<td>114±4*</td>
</tr>
<tr>
<td>Atropine injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Balloon inflation, max</td>
<td>155±8</td>
<td>163±6</td>
<td>181±6†</td>
<td>75±5‡</td>
<td>146±6*</td>
<td>66±2‡</td>
<td>161±9</td>
<td>180±4*</td>
<td>179±7</td>
</tr>
<tr>
<td>Atropine: therapeutic recovery</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Recovery</td>
<td>77±6</td>
<td>104±7*</td>
<td>65±8</td>
<td>55±2‡</td>
<td>67±4‡</td>
<td>50±3‡</td>
<td>92±6</td>
<td>97±6</td>
<td>121±2†§</td>
</tr>
</tbody>
</table>

Heart rate, bpm

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline values</td>
<td>433±16</td>
<td>421±17</td>
<td>362±16†</td>
<td>402±11</td>
<td>397±6</td>
<td>397±15</td>
<td>379±18§</td>
<td>395±12</td>
<td>370±4</td>
</tr>
<tr>
<td>l-NAME</td>
<td>—</td>
<td>386±12</td>
<td>—</td>
<td>355±6</td>
<td>—</td>
<td>—</td>
<td>360±11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Balloon insertion</td>
<td>387±13</td>
<td>366±12</td>
<td>341±21</td>
<td>364±28</td>
<td>327±11</td>
<td>415±41‡</td>
<td>412±14</td>
<td>342±17*</td>
<td>385±14§</td>
</tr>
<tr>
<td>Pentolinium injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>313±18</td>
<td>290±12</td>
<td>344±11</td>
<td>399±13</td>
<td>361±12</td>
<td>383±9</td>
</tr>
<tr>
<td>Atropine injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Balloon inflation, max</td>
<td>283±30</td>
<td>190±34*</td>
<td>169±25†</td>
<td>386±21‡</td>
<td>421±48‡</td>
<td>439±23‡</td>
<td>434±7§</td>
<td>434±12§</td>
<td>416±4§</td>
</tr>
<tr>
<td>Atropine: therapeutic recovery</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Recovery</td>
<td>357±19</td>
<td>328±11</td>
<td>294±32†</td>
<td>317±21</td>
<td>313±16</td>
<td>292±5</td>
<td>399±9</td>
<td>339±22*</td>
<td>366±4§</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Significant difference (P < 0.05; one-way ANOVA with post hoc LSD test). All F ratios and LSD values are presented in Table 3. *Influence of acute l-NAME administration vs. corresponding control (groups 2 vs. 1; 5 vs. 4; 8 vs. 7; 11 vs. 10). †Influence of chronic l-NAME administration vs. corresponding control (groups 3 vs. 1; 6 vs. 4; 9 vs. 7; 12 vs. 10). ‡Influence of pentolinium administration vs. corresponding control (groups 4 vs. 1; 5 vs. 2; 6 vs. 3). §Influence of atropine administration vs. corresponding control (groups 7 vs. 1; 8 vs. 2; 9 vs. 3).

R1113NITRIC OXIDE AND NEUROGENIC PULMONARY EDEMA

Table 2. Baseline mean arterial pressure and heart rate values, as well as the values found after particular surgical procedures in rats anesthetized with 1.5% isoflurane and subjected to acute or chronic l-NAME treatment and preventive pentolinium or atropine treatment

RESULTS

Spinal cord injury under different degrees of isoflurane anesthesia. The rapid inflation of the balloon in the spinal channel of animals anesthetized with 1.5% isoflurane anesthesia caused a considerable BP elevation, HR decrease, and severe NPE in all cases. The pulmonary index and the extent of subpleural bleeding (Table 1), as well as the BP rise and HR decrease (Tables 2 and 3, Fig. 1A) corresponded to the values observed previously for this model of severe NPE (19). Conversely, the same procedure performed under 3% isoflurane anesthesia did not promote NPE.

Effect of l-NAME administration in rats anesthetized with 1.5% isoflurane (groups 1–3). Acute l-NAME injection before the spinal cord injury was associated with 83% mortality following spinal cord compression. All of the animals died within 10 min after balloon inflation due to a rapid development of NPE. A few minutes before death, their breathing frequency started to increase slowly, and they began to develop a so-called “death rattle.” Subsequently, their ventilation stopped, and after several seconds, a large amount of gaseous blood came out of their nostrils, followed by the cessation of their heart beat. The amount of gaseous blood was substantially

Springs, CO). The competitive inhibitor of NOS l-NAME (Sigma, St. Louis, MO) was administered either acutely before the spinal cord injury (30 mg/kg iv, reported as “acute”) or for 4 wk before the injury procedure (40 mg·kg⁻¹·day⁻¹ in the drinking fluid, reported as “chronic”). To eliminate the influence of the sympathetic nervous system, we administered the ganglionic blocker pentolinium (5 mg/kg iv; Sigma) 2 min before balloon inflation. To limit the baroreflex-induced HR decrease, atropine (4 mg/kg iv; Sigma) was given 2 min before spinal cord injury. The hemodynamic values were obtained at the following time points: 1) the baseline value before the onset of surgery, 2) the maximum value after l-NAME administration, 3) the maximum value after balloon insertion, 4) the minimum value after pentolinium or atropine administration, 5) the maximum value after balloon inflation, 6) the value 2 min after balloon inflation, and 7) the value after 10 min of recovery. The BP rise and HR changes elicited by spinal cord compression were evaluated by subtracting the values found after balloon insertion (groups 1–3 and 10–12) from the peak values seen after balloon inflation. In groups subjected to pharmacological interventions (groups 4–6 and 7–9), we subtracted the values found after pentolinium or atropine administration from the corresponding peak values.

Statistical analysis. The pulmonary index, MAP, and HR are reported as mean values ± SE. One-way ANOVA with a post hoc least significant difference test was used for comparison among the individual groups.
larger than that observed in our 1.5% isoflurane model of severe NPE. The pulmonary index was significantly increased compared with the 1.5% isoflurane model (group 1) (Table 1). Acute l-NAME administration caused a moderate increase in BP (20 mmHg) accompanied by about a 10% decrease in HR in animals anesthetized with 1.5% isoflurane. In these rats (group 2), balloon insertion into the epidural space already caused a small BP elevation, which was seen not only in this group, but also in groups 5 and 8 (prior to pentolinium or atropine injection). Acute l-NAME administration attenuated the BP rise but augmented the bradycardia accompanying the BP rise occurring shortly after spinal cord compression in rats anesthetized with 1.5% isoflurane (group 2 vs. group 1) (Fig. 1B, Fig. 2).

Chronic l-NAME administration for 4 wk caused a moderate elevation of basal BP similar to that seen after acute l-NAME injection, but it affected the development of neurogenic pulmonary edema less than did acute l-NAME administration. Only 33% of the chronically l-NAME-treated animals died as a result of NPE, and their P index was significantly lower compared with those in rats subjected to acute l-NAME treatment (group 3 vs. group 2). However, the P index was not significantly increased compared with our 1.5% isoflurane model (group 1). BP elevation and HR decrease after spinal cord injury were comparable to that seen in the acutely l-NAME-treated rats (group 2) (Table 2). Nevertheless, the marked HR decrease accompanying BP elevation also promoted the development of severe NPE in chronically l-NAME-treated spinal cord-injured rats (group 3).

Effect of preventive pentolinium administration in rats anesthetized with 1.5% isoflurane (groups 4—6). Ganglionic blockade by pentolinium prevented the BP rise, bradycardia, and the

Fig. 1. The time course of mean arterial pressure (MAP; top) and heart rate (HR; bottom) during the entire surgical procedure, balloon compression lesion (arrowhead), and recovery period in representative animals from individual experimental groups. A: balloon compression spinal cord lesion in an animal anesthetized with 1.5% (model of neurogenic pulmonary edema) (group 1). B: balloon compression spinal cord lesion in an acutely l-NAME pretreated animal anesthetized with 1.5% isoflurane (group 2). C: balloon compression spinal cord lesion in an acutely l-NAME-pretreated animal anesthetized with 3% isoflurane (group 11). D: balloon compression spinal cord lesion in an acutely l-NAME-pretreated animal anesthetized with 1.5% isoflurane, pretreated with atropine (group 8).
development of neurogenic pulmonary edema in rats anesthe-
tized with 1.5% isoflurane (group 4), as observed previously
(18). Importantly, pretreatment with pentolinium also pre-
vented NPE development and bradycardia in rats treated
cutely or chronically with 1-NAME (groups 5 and 6) (Tables
1 and 2). In fact, in pentolinium-pretreated rats, the spinal cord
compression usually elicited a HR acceleration instead of the
typical bradycardia (Fig. 2). These results show the importance
of a HR decrease under the conditions of a BP increase for the
development of NPE.

Effect of preventive atropine injection in rats anesthetized
with 1.5% isoflurane (groups 7–9). The administration of
atropine 2 min before balloon inflation caused a significant BP
decrease prior to spinal cord injury without affecting the HR.
This pharmacological intervention completely prevented the
development of NPE in untreated animals (group 7), whereas
no significant effect of atropine pretreatment was observed in
rats pretreated with 1-NAME (group 8), in which both mortality and the P index
were significantly reduced compared with group 2 (Table 1).
On the other hand, atropine pretreatment prevented NPE de-
velopment in rats chronically treated with 1-NAME (group 9).
Importantly, in all of these groups atropine administration
prevented the HR decrease, but not the BP elevation, occurring early after balloon inflation (Table 2). The BP rise elicited by
spinal cord compression in atropine-pretreated rats tended to be
higher than in nonpretreated rats (groups 7–9 vs. groups 1–3)
(Fig. 2, Table 2). After atropine pretreatment, spinal cord
compression did not elicit the characteristic bradycardia, but
rather a borderline HR acceleration (Fig. 1D), which tended to
be smaller in atropine-pretreated animals (groups 7–9) than in
rats pretreated with pentolinium (groups 4–6) (Fig. 2).

Effect of 1-NAME administration in rats anesthetized with
3% isoflurane (groups 10–12). Neither acute nor chronic administration of 1-NAME promoted neurogenic pulmonary edema in animals anesthetized with 3% isoflurane. In addition, the values of the pulmonary index in rats treated with 1-NAME tended to be lower than those in animals without 1-NAME administration (groups 11 and 12 vs. group 10) (Table 3). Although pulmonary edema did not develop in any animal from the 3% isoflurane groups (irrespective of 1-NAME treat-
ment), the magnitude of the BP increase after balloon inflation seen in particular 3% isoflurane groups was comparable to the changes seen in the corresponding 1.5% isoflurane groups.
Nevertheless, HR did not decrease after balloon compression of the spinal cord in any of the 3% isoflurane groups (Table 2, Fig. 1C), indicating that baroreflex-induced bradycardia during the hypertensive reaction might be an important mechanism for
the development of NPE.

Finally, we demonstrated that the development of NPE
cannot be influenced by the therapeutic administration of
atropine (4 mg/kg iv) 2 min after balloon compression of the
spinal cord (group 13). The delayed atropine injection had no
positive effect on NPE development in rats with spinal cord
injury (P index 0.78 ± 0.04, n = 8). In this group, there was a similar BP rise (+51 ± 4 mmHg) and HR reduction
(−138 ± 29 bpm) after the balloon compression lesion as in
untreated rats subjected to spinal cord compression in 1.5%
isoﬂurane anesthesia (Table 3).

DISCUSSION

The present study confirmed our original findings (18) that
balloon compression of the thoracic spinal cord results in NPE
development in rats anesthetized with 1.5% isoflurane but not
in those anesthetized with 3% isoflurane. The inflation of a
balloon to 15 μl in the spinal channel caused a considerable BP
increase in both groups, but this BP rise was accompanied by
a HR reduction only in rats anesthetized with 1.5% isoflurane
(Fig. 2). The proposed importance of the sympathetic nervous
system (SNS) for the development of neurogenic pulmonary edema (12, 16) raised the question of whether the elimination
of nitric oxide would enhance the deleterious effects of SNS
activation on NPE development because NO is known to
attenuate peripheral sympathetic vasoconstriction (26) and to
inhibit central sympathetic tone (13).

Acute NOS inhibition worsened NPE development in the
1.5% isoflurane model (group 2 vs. group 1), whereas no
significant effect of NOS inhibition on NPE development was
observed in the 3% isoflurane model (groups 11 and 12 vs.
group 10). A similar deleterious effect of 1-NAME adminis-
tration on the severity and occurrence of NPE was reported by
Hamdy et al. (5), whereas increased NO production reduced the
severity of NPE in a similar model (8). The worsening of
NPE development in NO-deficient rats anesthetized with 1.5%
isoflurane was not associated with a greater BP rise, but there
was a clear-cut tendency toward a more pronounced HR
reduction after balloon inflation (Fig. 2). The mechanism
underlying the adverse effects of NO deficiency on NPE
development is probably based upon the lack of vasodilator
NO action counteracting a part of the sympathetic vasocon-
striction, so that a more profound baroreflex-induced bradycardia might occur (13, 26, 27). The most striking difference between NO-deficient rats subjected to 1.5% or 3% isoflurane anesthesia was the presence of baroreflex-induced bradycardia in the former animals, whereas no significant HR changes were observed after balloon compression of the spinal cord in rats anesthetized with 3% isoflurane (Fig. 2). This is in accordance with earlier findings that higher isoflurane concentrations decrease the baroreflex gain in the control of HR and sympathetic nerve activity (2, 11, 17).

The above findings suggest that the mechanism(s) of NPE development might involve sympathetic vasoconstriction leading to blood mobilization from the systemic circulation into the pulmonary vessels. The concomitant activation of the arterial baroreflex, which is absent in rats anesthetized with 3% isoflurane, causes such a decrease in HR that prevents effective blood pumping into the systemic circulation. Bradycardia has been recognized as an important factor contributing to NPE development in animals with intracranial hypertension (for a review, see Ref. 12). Nevertheless, bradycardia occurring after intracranial hypertension appears to be initiated by the respective centers in the medulla oblongata rather than by peripheral baroreceptor feedback (3). To test the role of bradycardia and its mechanism in our NPE model, we have successfully employed two different interventions to prevent a baroreflex-induced HR reduction following spinal cord compression. First, a ganglionic blocker, pentolinium, was used to prevent a sympathetic discharge leading to a BP increase and the consequent activation of the arterial baroreflex.

Pentolinium pretreatment indeed abolished the BP rise and bradycardia in both rats with preserved NO synthesis (group 4) and also chronically NO-deficient rats (group 6). On the other hand, a quite interesting response to spinal cord compression was found in pentolinium-pretreated rats subjected to acute NOS inhibition (group 5) (Fig. 2, Table 2). The mechanism of this surprising pressor response in acutely NO-deficient rats subjected to ganglionic blockade is not clear, but we can speculate about the involvement of other pressor agents (ANG II, endothelin-1, vasopressin) acting on the resistance vasculature in the absence of sympathetic vasoconstriction.

The second intervention used in our study was atropine pretreatment aimed at interrupting the cholinergic mechanisms mediating the baroreflex-induced bradycardia elicited after spinal cord compression. Preventive atropine administration not only abolished this bradycardia in both untreated and NO-deficient rats (groups 7–9) but also converted it into moderate tachycardia in both groups. This atropine pretreatment completely prevented the occurrence of NPE in both untreated and chronically NO-deficient animals anesthetized with 1.5% isoflurane (groups 7 and 9) and significantly attenuated NPE development in acutely NO-deficient rats subjected to the same anesthesia (group 8). Because therapeutic administration of atropine 2 min after balloon inflation (group 13) had no beneficial effect on NPE development, it is evident that an early bradycardic response after spinal cord compression is decisive for NPE pathogenesis.

**Perspectives and Significance**

Our data indicate that the development of neurogenic pulmonary edema in rats subjected to moderate anesthesia (1.5% isoflurane) is dependent upon a marked decrease of HR under the conditions of high BP elicited by the activation of the sympathetic nervous system. This vicious circle can be inter-

---

**Table 3. Baseline mean arterial pressure and heart rate values, as well as the values found after particular surgical procedures in rats anesthetized with 3% isoflurane and subjected to acute or chronic L-NAME treatment or in rats anesthetized with 1.5% isoflurane subjected to therapeutic atropine administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>Isoflurane</th>
<th>Pharmacological intervention</th>
<th>3% acute L-NAME</th>
<th>3% chronic L-NAME</th>
<th>1.5% T-atropine</th>
<th>Mean arterial pressure, mmHg</th>
<th>Heart rate, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Baseline values</td>
<td>78±4**</td>
<td>85±7</td>
<td>115±7†</td>
<td>96±2</td>
<td>15.3</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>t-NAME</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>103±8</td>
<td>2.0</td>
</tr>
<tr>
<td>12</td>
<td>Balloon insertion</td>
<td>84±4</td>
<td>90±5**</td>
<td>97±10**</td>
<td>102±4</td>
<td>9.2</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>Pentolinium injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Atropine injection</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>15.0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Balloon inflation, max</td>
<td>127±8**</td>
<td>147±4*</td>
<td>155±6**</td>
<td>155±3</td>
<td>25.3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Atropine: therapeutic</td>
<td>—</td>
<td>—</td>
<td>90±3</td>
<td>—</td>
<td>10.1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>60±2**</td>
<td>88±10*</td>
<td>79±6†</td>
<td>73±4</td>
<td>10.1</td>
<td>17</td>
</tr>
</tbody>
</table>

*Influence of acute L-NAME administration vs. corresponding control (groups 2 vs. 1; 5 vs. 4; 8 vs. 7; 11 vs. 10). †Influence of chronic L-NAME administration vs. corresponding control (groups 3 vs. 1; 6 vs. 4; 9 vs. 7; 12 vs. 10). **Difference between 1.5% and 3% isoflurane anesthesia (groups 10 vs. 1; 11 vs. 2; 12 vs. 3).
ruptured either by ganglionic blockade (attenuating the sympathetic discharge) or by the blockade of cholinergic muscarinic receptors (eliminating the HR reduction). The above hemodynamic alterations are especially pronounced in rats subjected to acute NO synthase inhibition. Nitric oxide has a partial protective effect on NPE development because it attenuates sympathetic vasoconstriction, BP rise, and subsequent baroreflex-induced bradycardia following spinal cord injury. Further research should elucidate the therapeutic potential of atropine and/or other parasympatholytic agents in other (less severe or slowly developing) models of NPE.

ACKNOWLEDGMENTS

We thank to Pavlína Macková for her excellent technical assistance. We thank James Dutt for critical reading of the manuscript.

GRANTS

We acknowledge the support provided by the Grants AV0Z50390512, AV0Z50110509, 1M0538, LC554, and 1M0510 by the Ministry of Education of the Czech Republic, Grants GACR 369/06/61246 and GACR 305/08/0139 by Grant Agency of the Czech Republic, Grants IGA MZ 1A8697-5 and IGA MZ NR/8339-3 by Czech Ministry of Health, Grant IAA 500390902 by Internal Agency of the Academy of the Czech Republic and the European Comission FP6 project RESCUE (LSHB-CT-2005-518233).

REFERENCES