The role of sympathetic nervous system in the development of neurogenic pulmonary edema in spinal cord-injured rats

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The role of sympathetic nervous system in the development of neurogenic pulmonary edema (NPE) in rats with balloon compression of spinal cord. J Appl Physiol 112: 1–8, 2012. First published September 8, 2011; doi:10.1152/japplphysiol.00574.2011.—The pronounced activation of sympathetic nervous system is a necessary prerequisite for the development of neurogenic pulmonary edema (NPE) in rats with balloon compression of spinal cord. In this study we examined whether this is a consequence of rapid activation of spinal pathways leading to sympathetic vasoconstriction, blood pressure rise, and reflex bradycardia. We found that NPE development can be prevented by epidural upper thoracic anesthesia or by transection of the upper spinal cord. This indicates an important role of spinal pathways activation. NPE development can also be prevented by moderate blood loss, supporting the role of blood redistribution to pulmonary circulation. In rats developing NPE the catecholamine surge following spinal cord compression involved not only a dramatic increase of circulating norepinephrine but also of epinephrine levels. The pretreatment of rats with α-1 adrenoceptor blocker prazosin, α-2 adrenoceptor blocker yohimbine, or calcium channel blocker nifedipine prevented NPE development, whereas the effect of β-adrenoceptor blockade with propranolol was less convincing. In conclusion, considerable activation of thoracic spinal pathways, followed by marked catecholamine secretion, play a major role in the development of NPE in spinal cord-injured rats. Enhanced α-adrenergic nifedipine-sensitive vasoconstriction is responsible for observed blood pressure changes, subsequent baroreflex bradycardia, and blood volume redistribution, which represent major pathogenetic mechanisms of NPE development.

α-adrenergic vasoconstriction; baroreflex bradycardia; epidural anesthesia; spinal pathways activation; blood volume redistribution

NEUROGENIC PULMONARY EDEMA (NPE) has been described as an acute life-threatening complication following several central nervous system injuries, including spinal cord injury, subarachnoid hemorrhage, primary spinal cord hemorrhage, brain trauma, intracerebral bleeding, severe epileptic grand mal seizure, or subdural hematoma (1, 4–6, 9, 14, 19, 28, 29). It develops rapidly following the injury and significantly complicates the overall clinical status of the patient. It is characterized by marked pulmonary vascular congestion with perivascular edema, extravasation and intra-alveolar accumulation of protein-rich edema fluid, and intra-alveolar hemorrhage (7, 11, 12). Both the release of vasoactive substances and a severe transient sympathetic discharge are thought to participate in this process. Several experimental studies have indicated that the neurons responsible for this severe sympathetic discharge are located in nuclei of hypothalamus and medulla oblongata. These centers represent so-called NPE trigger zones (1). The overactivation of the sympathetic nervous system is associated with the enhanced secretion of catecholamines from peripheral sympathetic nerve endings, which leads to peripheral vasoconstriction, an increase in systemic vascular resistance and blood pressure together with the augmentation of central blood volume, and a reduction in the compliance of the left ventricle. In the periphery, these changes are followed by the constriction of the pulmonary veins, an increase in pulmonary capillary hydrostatic pressure, damage to the alveolar wall, and the leakage of fluid into the interstitium and intra-alveolar space, and hemorrhage resulting in the typical picture of NPE (1, 5). Despite these reports, the exact cascade leading to the development of NPE is still unclear (11, 12).

We have recently demonstrated (27) that major activation of sympathetic nervous system together with pronounced baroreflex-induced bradycardia are necessary prerequisites for the development of severe NPE, which is experimentally induced by rapid epidural balloon compression of the thoracic spinal cord in rats anesthetized by 1.5% isoflurane in air (21, 22). On contrary, the same procedure did not lead to NPE when deeper anesthesia with 3% isoflurane was employed (21). Our experiments also indicated that the prevention of any of these two decisive components—blood pressure elevation by ganglionic blocker pentolinium and/or reflex bradycardia by atropine pretreatment—abolished NPE development following spinal cord compression (27). This is in line with the fact that isoflurane exerts sympathoinhibitory effects (20) and attenuates baroreflex gain in the control of either sympathetic nerve activity (20) or heart rate (HR) (13).

The aim of the present study was to evaluate the importance of the activation of ascending spinal pathways, elicited by rapid compression of thoracic spinal cord, for sympathetic hyperactivity and NPE occurrence. To achieve this goal, we have used two different approaches: upper spinal cord transection and epidural anesthesia above the compression site to interrupt neural signal transmission. It should be mentioned that enhanced sympathetic vasoconstriction occurring after spinal cord compression is manifested not only by blood pressure elevation but also by increased venous return due to intensive visceral vasoconstriction redistributing blood to pulmonary circulation. This situation is further aggravated by the difficulties of the heart to pump the blood into systemic circulation during reflex bradycardia. Thus the second aim of our study was to evaluate whether moderate blood volume reduction just before spinal compression might have protective effects on NPE development.

The third aim of this study was to assess the contribution of particular components of sympathetic nervous system to NPE development. A special attention was paid to vasoconstriction...
mediated by α-adrenoceptors and subsequent calcium entry through voltage-dependent Ca\(^{2+}\) channels of L type (L-VDCC, sensitive to dihydropyridine Ca\(^{2+}\) antagonists). We were also interested to know whether the enhanced stimulation of β-adrenoceptors by released norepinephrine and/or epinephrine could attenuate NPE development through its vasodilatory and cardiac effects.

Finally, we examined the changes of circulating norepinephrine and epinephrine following spinal cord compression in animals subjected either to different degree of isoflurane anesthesia (1.5% vs. 3%) or to epidural anesthesia.

**MATERIALS AND METHODS**

**Animals.** We used 91 male Wistar rats (Velaz, Prague, Czech Republic) with body weights between 300 and 330 g. This study was performed in accordance with the European Communities Council Directive of 24th of November 1986 (86/609/EEC) regarding the use of animals in research and was approved by the Ethical Committee of the Institute of Physiology, Academy of Sciences of 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 anesthetized with either 1.5% or 3% isoflurane in air (flow 300 ml/min), and an arterial catheter for the monitoring of blood pressure and HR as well as a venous catheter for the administration of vasoactive drugs (prazosin, nifedipine, propranolol, or yohimbine) were inserted into the common carotid artery and internal jugular vein, respectively. In particular groups, different interventions were performed before spinal cord compression: 1) transaction of the upper spinal cord, 2) the insertion of catheter into upper thoracic region for trimecaine epidural anesthesia, or 3) moderate blood loss (3 ml, i.e., ~15% of blood volume) from carotid artery. In four animals, the balloon was inserted into the lumbar (L) instead of the thoracic (T) spinal level. Thereafter the animal was put in a prone position, and the balloon compression was performed. Rats were euthanized 15 min after lesioning (i.e., 10 min after balloon deflation). The grade of NPE was independently evaluated using macroscopic visual examination of subpleural bleeding and the p-index (lung weight/body weight × 100). Controls were healthy noninjured animals, euthanized immediately after the induction of anesthesia. The possible role of isoflurane per se in inducing NPE was excluded in our previous study (21). For the detailed list of experimental groups see Table 1.

**Balloon compression spinal cord lesion.** To induce a spinal cord injury, we used the model of an epidural balloon compression lesion (32), as described previously (21). Briefly, under aseptic conditions, a 2-cm median skin incision at the T10–L1 level was made. The dorsal muscles were shifted laterally, and the T10 and T11 spinous processes were removed. A hole was drilled into the T10 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-μl Hamilton syringe, was inserted into the dorsal epidural space 10 mm rostrally, to reach the T8–T9 spinal level. Using a micromanipulator, the balloon was rapidly inflated to 15 μl, and the inflated balloon was left in place for 5 min. Subsequently, the balloon was deflated and removed. Rats were euthanized 10 min later.

The immediate inflation of the balloon in the epidural space of the thoracic spinal channel with 15 μl water under 1.5% isoflurane anesthesia reliably and reproducibly produces severe NPE (21, 22). In the present experiments we therefore studied the effects of different surgical or pharmacological procedures on the development of NPE in this model (1.5% model).

**Epidural anesthesia.** After the induction of anesthesia and insertion of arterial catheter for the monitoring of blood pressure and HR, the animals were put into prone position. The spinal cord was reached by exactly the same procedure as described for balloon compression. A thin catheter (PE10) was inserted into epidural space to reach the spinal T2 level. Subsequently, the wound site was hermetically closed by Histoacryl, with a particular care not to disturb the spinal cord tissue. Subsequently, 100 μl of trimecaine (Mesocain, 10 mg/ml) was administered. This dose of trimecaine was chosen on the basis of our preliminary experiments, in which 100 μl of trimecaine produced a temporary paraplegia of both hindlimbs for at least 30 min (unpublished data).

**Upper thoracic spinal cord transaction.** After the induction of anesthesia and insertion of arterial catheter for the monitoring of blood pressure and HR, the animals were put into prone position. The upper thoracic spinal cord was reached by exactly the same procedure as described for balloon compression, but the targeted spinal level was T4. After that, the surgical approach for the balloon compression lesion was prepared. First, sharp rapid spinal cord transaction at the T4 spinal level was performed, and 3 min later, balloon compression lesion at the T4 level was performed as described previously.

**Lumbar spinal cord compression.** After the induction of anesthesia and insertion of arterial catheter for the monitoring of blood pressure and HR, the animals were put into prone position. The surgical approach to the spinal cord was performed as described for thoracic spinal cord compression. Subsequently, the 2F French Fogarty catheter was inserted into the dorsal epidural space 8 mm caudally, to

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>Isoflurane</th>
<th>n</th>
<th>Absent (% of 2n)</th>
<th>Grade I (% of 2n)</th>
<th>Grade II (% of 2n)</th>
<th>Grade III (% of 2n)</th>
<th>p-index</th>
<th>Died (%)</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.5%</td>
<td>9</td>
<td></td>
<td>1 (6%)</td>
<td>17 (94%)</td>
<td></td>
<td>0.78 ± 0.05*</td>
<td>3* (33%)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3%</td>
<td>8</td>
<td>16 (100%)</td>
<td></td>
<td></td>
<td></td>
<td>0.50 ± 0.06</td>
<td></td>
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<tr>
<td>3</td>
<td>Epidural anesthesia</td>
<td>1.5%</td>
<td>10</td>
<td>20 (100%)</td>
<td>0.44 ± 0.01</td>
<td>0.46 ± 0.04</td>
<td>0.42 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>4</td>
<td>Upper spinal cord transection</td>
<td>1.5%</td>
<td>6</td>
<td>12 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>5</td>
<td>Prazosin (1 mg/kg)</td>
<td>1.5%</td>
<td>6</td>
<td>12 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>6</td>
<td>Yohimbine (1 mg/kg)</td>
<td>1.5%</td>
<td>6</td>
<td>12 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>7</td>
<td>Nifedipine (0.4 mg/kg)</td>
<td>1.5%</td>
<td>4</td>
<td>8 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>8</td>
<td>Propranolol (1–4 mg/kg)</td>
<td>1.5%</td>
<td>20</td>
<td>16 (80%)</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
<td>0.52 ± 0.02*</td>
<td>0.47 ± 0.03</td>
<td>0.47 ± 0.03</td>
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<tr>
<td>9</td>
<td>Blood loss (3 ml)</td>
<td>1.5%</td>
<td>5</td>
<td>10 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>Lumbar compression</td>
<td>1.5%</td>
<td>4</td>
<td>8 (100%)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.03</td>
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<tr>
<td>11</td>
<td>Intact controls</td>
<td>1.5%</td>
<td>13</td>
<td>26 (100%)</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.02</td>
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The p-index is given as mean values ± SE; n = no. of rats and 2n = no. of lungs. Since there were no significant dose-dependent differences in hemodynamic or pulmonary effects of spinal cord compression, all 3 subgroups of propranolol-treated rats were merged together. The absence or presence of subpleural bleeding (evaluated as Grades I—III) in different groups are shown as follows. The total number of lungs in each group; the right and left lung were considered separately, and the percentage of affected lungs was calculated in terms of all lungs in the corresponding group. *Significant differences (P < 0.05) from control group. The occurrence of death is shown as the total number of deaths in each group and as the percentage of all animals in the group. Control animals are animals without spinal cord injury, sacrificed immediately after the onset of anesthesia. n = no. of rats and 2n = no. of lungs.
reach the L₂–L₅ spinal level. Using a micromanipulator, the balloon was rapidly inflated to 15 μl, and the inflated balloon was left in place for 5 min. Subsequently, the balloon was deflated and removed. Rats were euthanized 10 min later.

**Monitoring of circulating levels of epinephrine and norepinephrine.** The immediate inflation of the balloon in the epidural space of the thoracic spinal channel with 15 μl water under 1.5% isoflurane anesthesia reliably and reproducibly produces severe NPE, whereas the animals that undergo the same procedure under 3% isoflurane anesthesia do not develop NPE (21, 22). In the present experiments we therefore compared catecholamine surge in these two contrasting models. Blood samples were taken before spinal surgery, immediately after the inflation of the balloon in spinal channel, and at the end of the recovery period. Serum concentrations of epinephrine and norepinephrine were determined using commercial 2-CAT RIA kit (LDN, Hamburg, Germany) after extraction and acylation of samples. The sensitivity was 0.03 ng/ml for both epinephrine and norepinephrine.

As a control group, we used animals where both adrenal glands were removed using extraperitoneal approach, i.e., through small incisions made in a midscapular line at the lumbar level. The sensitivity was 0.03 ng/ml for both epinephrine and norepinephrine.

**Measurement of blood pressure and HR changes.** Mean arterial pressure (MAP, mmHg) and HR (beats/min) were monitored for at least 5 min before the spinal surgical procedure, throughout the entire procedure, and for 15 min after the balloon compression of the spinal cord using a PowerLab system (ADInstruments, Colorado Springs, CO). In particular groups, we pretreated the rats with one of following drugs: 1) α-1 adrenergic receptor blocker prazosin (1 mg/kg iv), 2) α-2 adrenergic receptor blocker yohimbine (1 mg/kg iv), 3) β-adrenergic receptor blocker propranolol (1, 2, and 4 mg/kg iv), and 4) calcium channel blocker nifedipine (0.4 mg/kg iv). These drugs were given after balloon insertion, i.e., shortly before spinal cord compression, which was carried out after a temporary stabilization of blood pressure was achieved. The hemodynamic values were obtained at the following time points: 1) the baseline value before the onset of surgery, 2) the stabilized values before balloon inflation, 3) the maximum value after balloon inflation, 4) the value 2 min after balloon inflation, and 5) the value at the end of recovery (10 min after balloon deflation).

MAP and HR changes elicited by spinal cord compression were evaluated by subtracting the values found after balloon insertion (groups 1–3 and 9–11) from the peak values seen after balloon inflation. In groups subjected to pharmacological interventions (groups 3 and 5–8), we subtracted the values found after drug administration from the corresponding peak values.

**Evaluation of NPE.** The lungs were immediately removed from euthanized animal and weighed by an independent investigator. To estimate the liquid accumulation in the lungs, the relative pulmonary weight was calculated as the pulmonary index (lung weight/body weight × 100), which has been previously demonstrated to be a very sensitive indicator of the degree of pulmonary edema (11, 12, 16, 21, 22). In all cases, a mild hematoma, maximally 1 mm in diameter, was found in the hilus area due to 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 (21, 22). 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 (21, 22, 25); thus the histology was not performed in the current experiments.

**Statistical analysis.** The pulmonary index, MAP, HR, and catecholamine levels are reported as mean values ± SE. One-way ANOVA with a post hoc least significant difference (LSD) test was used for the comparison among individual groups.

**RESULTS**

The effect of various spinal procedures and blood loss on NPE development. The rapid inflation of the balloon in the spinal channel of rats anesthetized with 1.5% isoflurane anesthesia (Table 1, group 1) caused a considerable blood pressure elevation, baroreflex-induced HR decrease, and severe NPE in all cases (Table 1 and Figs. 1 and 2). The pulmonary index and the extent of subpleural bleeding as well as the blood pressure rise and HR decrease corresponded to the values observed previously for this model of severe NPE. Conversely, the same procedure performed under 3% isoflurane anesthesia (group 2) did not promote NPE. In this group, the changes elicited by spinal cord compression were ΔMAP = 72 ± 9 mmHg and ΔHR = 4 ± 3 beats/min. These findings also confirmed our previous results (21).

None of the animals pretreated with upper thoracic transection or epidural anesthesia developed NPE. Neither p-index nor the extent of subpleural bleeding differed significantly from intact controls (Table 1, groups 3 and 4). The epidural anesthesia prevented the blood pressure rise as well as HR decrease in these animals (Figs. 1 and 2). Similar protection against NPE was also observed in animals subjected to a moderate preventive blood loss (Table 1, group 9). Blood loss caused a

![Fig. 1. Changes in mean arterial pressure (MAP) and heart rate (HR) elicited by spinal cord compression in rats anesthetized with 1.5% isoflurane and subjected to either epidural anesthesia (group 3), upper thoracic spinal cord transection (group 4), or blood loss (group 9). Data are presented as means ± SE. Significant differences (P < 0.05) vs. control group (1.5% model of neurogenic pulmonary edema (NPE), group 1) are marked by asterisks.](http://jap.physiology.org/)
considerable blood pressure reduction before balloon inflation (Table 2). In these animals spinal cord compression elicited exaggerated blood pressure rise but no significant change in HR (Figs. 1 and 2).

NPE did not occur in rats in which spinal cord was compressed at the lumbar level (Table 1, group 10; ΔMAP $-25 \pm 8$ mmHg; ΔHR $-4 \pm 13$ beats/min; p-index = 0.43 ± 0.01). This is in accordance with our previous findings that different types of spinal cord lesion, such as lower thoracic spinal transection at the T8 level (Fig. 3). In animals with severe NPE, which were anesthetized with 1.5% isoflurane (group 1: 1.5% model), we have observed an 18-fold increase in serum epinephrine concentration and a 64-fold increase in serum norepinephrine concentration immediately after balloon compression. This increase of circulating catecholamine levels was normalized at the end of the recovery period. In rats anesthetized with 3% isoflurane (group 2), which did not develop NPE, there was a 40-fold increase in the level of serum norepinephrine but no significant change in epinephrine level after balloon compression of the spinal cord (Fig. 3). It is important to note that the levels of catecholamines were not increased during spinal cord compression in animals pretreated with upper thoracic epidural anesthesia (Fig. 3).

**Circulating catecholamines and NPE development.** To examine the role of sympathetic nervous system in NPE, we measured serum levels of epinephrine and norepinephrine before, during, and after the balloon compression spinal cord lesion (Fig. 3). In animals with severe NPE, which were anesthetized with 1.5% isoflurane (group 1: 1.5% model), we have observed an 18-fold increase in serum epinephrine concentration and a 64-fold increase in serum norepinephrine concentration immediately after balloon compression. This increase of circulating catecholamine levels was normalized at the end of the recovery period. In rats anesthetized with 3% isoflurane (group 2), which did not develop NPE, there was a 40-fold increase in the level of serum norepinephrine but no significant change in epinephrine level after balloon compression of the spinal cord (Fig. 3). It is important to note that the levels of catecholamines were not increased during spinal cord compression in animals pretreated with upper thoracic epidural anesthesia (Fig. 3).

**The contribution of α- and β-adrenergic mechanisms to the development of NPE.** The pretreatment of rats with a competitive inhibitor of α-1 adrenergic receptors prazosin (group 5), a competitive inhibitor of α-2 adrenergic receptors yohimbine (group 6), or calcium channel blocker nifedipine (group 7) prevented the NPE development, as indicated by unaltered p-index values (Table 1). All three blockers lowered blood pressure before spinal cord compression without significant changes in HR (Table 2). Figure 4 shows that all these pharmacological interventions prevented significant HR reduction, whereas blood pressure rise was attenuated by prazosin pretreatment only. These results suggest that α-adrenergic neurotransmission and consequent calcium influx through volt-
Age-dependent calcium channels play a crucial role in NPE development. Moreover, the data presented in Fig. 4 clearly confirm our above mentioned results from experiments with different spinal cord manipulations that marked rapid HR decrease, but not blood pressure rise, induced by spinal cord compression is essential for the development of NPE. On the other hand, a \( \alpha \)-2-adrenoceptor blocker yohimbine, or calcium channel blocker nifedipine prevented NPE development, whereas the effect of \( \beta \)-adrenoceptor blockade with propranolol was less convincing. Thus a considerable activation of thoracic spinal pathways leading to enhanced \( \alpha \)-adrenergic vasoconstriction (mediated by calcium entry via L-VDCC) plays a major role in the development of NPE in spinal cord-injured rats. Our experiments confirmed the decisive role of baroreflex-induced bradycardia in the pathogenesis of NPE following spinal cord compression in particular experimental groups (prazosin, yohimbine, propranolol, nifedipine) of rats anesthetized with 1.5% isoflurane. These hemodynamic changes elicited by spinal cord compression were similar at all three propranolol doses (data not shown). It is evident that the degree of protection against NPE induced by different interventions on sympathetic nervous system clearly corresponds to the extent of attenuation of HR rate decrease occurring after spinal cord injury.

**DISCUSSION**

The pronounced activation of sympathetic nervous system is a necessary prerequisite for the development of NPE in rats with the balloon compression of spinal cord. In this study we found that NPE development can be prevented by epidural upper thoracic anesthesia or by upper transection of the thoracic spinal cord. This indicates an important role of rapid activation of spinal pathways for the enhancement of the sympathetic outflow. NPE development can also be prevented by a moderate blood loss, supporting the role of blood redistribution to pulmonary circulation. In rats developing NPE the catecholamine surge following spinal cord compression involved not only a dramatic rise of circulating norepinephrine but also a major increase of epinephrine levels. The pretreatment of rats with \( \alpha \)-1 adrenoceptor blocker prazosin, \( \alpha \)-2 adrenoceptor blocker yohimbine, or calcium channel blocker nifedipine prevented NPE development, whereas the effect of \( \beta \)-adrenoceptor blockade with propranolol was less convincing. Thus a considerable activation of thoracic spinal pathways leading to enhanced \( \alpha \)-adrenergic vasoconstriction (mediated by calcium entry via L-VDCC) plays a major role in the development of NPE in spinal cord-injured rats. Our experiments confirmed the decisive role of baroreflex-induced bradycardia in the pathogenesis of NPE following spinal cord compression.
compression, because all experimental procedures eliminating HR reduction after spinal cord compression also abolished NPE development.

**Pronounced rapid activation of thoracic spinal pathways is essential for NPE occurrence.** Our study showed that NPE development can be prevented by preceding epidural upper thoracic anesthesia with tramadol. In human medicine, this intervention is routinely used to completely anesthetize the central as well as peripheral spinal neural pathways to perform particular surgical approaches, such as hip joint surgery or obstetric operations. We used this procedure in our NPE model to examine the effect of complete abolishing of the activation of spinal neural pathways. Our results indicate that rapid balloon compression of anesthetized lower thoracic spinal cord completely prevented the massive activation of NPE trigger zone afferent pathways (for review on NPE trigger zone see Refs. 1, 23, 24). In our study this was reflected by the lack of characteristic hemodynamic response associated with NPE development as well as by the absence of catecholamine surge. Moreover, the balloon compression of anesthetized thoracic spinal cord was not accompanied by skeletal muscle contractions typical for NPE model (21, 22), indirectly supporting the efficiency of epidural anesthesia. Similar preventive effects on NPE development were achieved by tranSECTION of the spinal cord at the T1 level.

Interestingly, the hyperactivation of spinal neural pathways, supposed in the compressive model, does not seem to have a role in other spinal cord injury models, such as spinal transection or hemisection (22, 24, 25). Moreover, only rapid but not slow compression of spinal cord elicited NPE under otherwise same experimental design (25). Many clinical experiences indicate that the moment of sudden severe hyperactivity of spinal or cerebral pathways clearly corresponds to the time when the NPE was elicited (for review see Refs. 1, 5). Thus both the extent as well as the rate of spinal neural hyperactivation might be crucial for the development of NPE. It has been published previously that cerebral compression elicited systemic arterial hypertension and pulmonary edema. These responses were prevented by spinal transection at C7 level, but not by decerebration (2, 3). On the other hand, we observed that NPE did not develop when the balloon compression was performed at the lumbar instead of lower thoracic spinal level. This is probably the result of absence of sympathetic nerve fibers at the lumbar level of the spinal cord.

**Blood volume redistribution plays an important role in NPE development.** The redistribution of blood from splanchnic regions to pulmonary circulation due to pronounced sympathetic vasoconstriction seems to be the mechanism of the increase of central blood volume in NPE, which is characterized not only by vascular congestion but also by blood extravasation. To evaluate the contribution of this hemodynamic factor in the pathogenesis of NPE we have induced a moderate blood loss (~15% volume of circulating blood) before spinal cord compression. It is evident that this intervention successfully prevented NPE development, indicating the importance of augmented venous return and overfilling of pulmonary circulation during early stages of NPE development, when baroreflex-induced reduction of HR impairs actual cardiac performance (27).

Moreover, it should be considered whether a higher degree of isoflurane anesthesia (3% instead of 1.5%) might be associated with a greater blood deposition in splanchnic vessels, because isoflurane was reported to attenuate also the tone of capacitance-regulating mesenteric veins (30). Thus the protective effects exerted by moderate blood loss on NPE development might share similar mechanisms with those of a more pronounced isoflurane anesthesia.

Norepinephrine-induced L-VDCC opening is a necessary prerequisite for NPE development. Balloon compression of the spinal cord is accompanied by a major catecholamine surge, which can be prevented not only by epidural anesthesia (Fig. 3) but also by bilateral adrenalectomy (Šedý J, Zicha J, unpublished data). The elevation of circulating norepinephrine was associated with high epinephrine levels only in rats anesthetized with 1.5% isoflurane, which developed NPE, but not in those anesthetized with 3% isoflurane (Fig. 3). The crucial role of high epinephrine levels in the development of pulmonary edema has been previously shown in experiments of Maron and coworkers, who observed dose-dependent relationship between epinephrine plasma levels and alveolar liquid clearance, leading to pulmonary edema (10, 15).

We have earlier demonstrated that blood pressure rise caused by the activation of sympathetic nervous system after spinal cord compression can be successfully prevented by ganglionic blockade (using pentolinium pretreatment) (21). In this study we have tried to evaluate the importance of particular components of adrenergic vasoconstriction in the pathogenesis of NPE. To achieve this goal we have pretreated our rats with either α1-adrenoceptor blocker prazosin or α2-adrenoceptor blocker yohimbine. Our data (Fig. 4, Table 1) indicate that both types of α-adrenoceptor blockade were able to prevent not only NPE occurrence but also HR reduction which is a hemodynamic change essential for NPE development (26, 27). The similar effects of α1- and α2-adrenoceptor blockade are not so surprising if we consider that an extensive catecholamine stimulation of these two different types of α-adrenoceptors always leads to a major augmentation of calcium entry through L type voltage-dependent Ca2+ channels (L-VDCC) (8, 17, 18). In the first case, the activation of α1-adrenoceptors causes severe depletion of intracellular calcium stores via inositol trisphosphate stimulation of its receptors on sarcoplasmic reticulum. Subsequent opening of store-operated Ca2+ channels (SOC) to replete calcium stores is associated with enhanced Na+ entry leading to membrane depolarization followed by L-VDCC opening. In the second case, the activation of α2-adrenoceptors stimulates the inhibitory influence of Gi proteins on adenylylate cyclase leading to decreased cAMP formation. Reduction of cAMP levels is associated with the opening of L-VDCC (probably due to diminished activity of large-conductance Ca2+-activated K+ channels the function of which is to hyperpolarize cell membrane).

The fundamental importance of calcium entry via L-VDCC for the development of NPE was demonstrated in our experiments in which the pretreatment of rats with nifedipine (dihydropyridine antagonist of L-VDCC) before spinal cord compression completely prevented the above mentioned HR reduction and NPE development (Fig. 4, Table 1). It should be pointed out that in our study nifedipine pretreatment had very similar effects as the pretreatment with either α1- or α2-adrenoceptor blockers.

Our results are also fully compatible with the earlier findings on isoflurane-induced hyperpolarization of smooth muscle...
cells in small resistance arterioles or capacitance venules of the rat (30, 31). The in situ superfusion of small mesenteric vessels with isoflurane caused membrane hyperpolarization through both neural (inhibition of sympathetic neural input) and non-neuronal mechanisms (inhibition of cAMP pathways and K⁺ channels). This hyperpolarization helps to close L-VDCC opened by sympathetic stimulation. Thus the deeper isoflurane anesthesia attenuated both blood pressure rise (by decreasing arterial constriction) and pulmonary venous return (by increasing splanchic capacity to deposit circulating blood).

On the other hand, our attempt to aggravate NPE development by β-adrenergic blockade with propranolol was not successful (Table 2). Our idea was to attenuate β-adrenergic vasodilatation (to increase blood pressure rise) and to diminish cardiac contractility (to reduce cardiac pumping of blood through the lungs). Surprisingly, our experiments with propranolol pretreatment revealed a moderate attenuation of NPE development instead of its exacerbation. This partial protective effect could be explained by HR reduction induced by propranolol administration before spinal cord compression (Table 2) that consequently elicited a substantially smaller HR decrease (Table 1).

Conclusions. Our study demonstrated that the rapid activation of ascendent spinal pathways is responsible for sympathetic hyperactivation occurring after spinal cord compression. Our data indicate that the blockade of enhanced α-adrenergic vasoconstriction (mediated by increased calcium entry through L-type of voltage-dependent Ca²⁺ channels) prevents NPE development. The present findings also support the essential role of baroreflex-induced HR reduction for the induction of NPE.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


