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RAPID COMMUNICATION

Neurogenic Pulmonary Edema Induced by Spinal Cord Injury in Spontaneously Hypertensive and Dahl Salt Hypertensive Rats

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Summary

Neurogenic pulmonary edema (NPE), which is induced by acute spinal cord compression (SCC) under the mild (1.5 %) isoflurane anesthesia, is highly dependent on baroreflex-mediated bradycardia because a deeper (3 %) isoflurane anesthesia or atropine pretreatment completely abolished bradycardia occurrence and NPE development in rats subjected to SCC. The aim of the present study was to evaluate whether hypertension-associated impairment of baroreflex sensitivity might exert some protection against NPE development in hypertensive animals. We therefore studied SCC-induced NPE development in two forms of experimental hypertension – spontaneously hypertensive rats (SHR) and salt hypertensive Dahl rats, which were reported to have reduced baroreflex sensitivity. SCC elicited NPE in both hypertensive models irrespective of their baroreflex sensitivity. It is evident that a moderate impairment of baroreflex sensitivity, which was demonstrated in salt hypertensive Dahl rats, does not exert sufficient protective effects against NPE development.

Key words

Hypertension • Rat • Neurogenic pulmonary edema • SHR • Dahl rats

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Neurogenic pulmonary edema (NPE) is an acute life-threatening complication following spinal cord or brain injury (Fontes *et al.* 2003). It is characterized by marked pulmonary vascular congestion, extravasation of protein-rich edema fluid and intraalveolar hemorrhage (Kondo *et al.* 2004, Kandatsu *et al.* 2005, Leal Filho *et al.* 2005a,b). Many pathophysiological mechanisms have been implicated in the development of neurogenic pulmonary edema, but the exact cascade leading to its development is still unclear (Leal Filho *et al.* 2005a,b). Both the release of vasoactive substances and a severe transient sympathetic discharge are thought to participate in this process (Taoka and Okajima 1998, Urdaneta and Layon 2003). These processes lead to 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 intraalveolar space (Fontes *et al.* 2003).

Our recent studies (Šedý *et al.* 2009a, 2011) confirmed the importance of major sympathetic discharge in the pathogenesis of severe NPE elicited by rapid epidural balloon compression of the thoracic spinal cord of rats, which were anesthetized by 1.5 % isoflurane (Šedý *et al.* 2007). Particular procedures preventing the rapid activation of the sympathetic nervous system (SNS) such as ganglionic blockade (Šedý *et al.* 2007, 2009a), slow gradual inflation of the balloon (Šedý *et al.* 2009b) as well as epidural anesthesia or transection of the spinal cord above the level of spinal cord compression (Šedý *et al.* 2011) prevented NPE development. Blood pressure rise occurring after spinal cord compression (SCC) is followed by a pronounced baroreflex-mediated bradycardia, which is of critical importance for NPE development. Indeed, bradycardia prevention by atropine pretreatment (Šedý *et al.* 2009a) or deeper anesthesia (3 % isoflurane) (Šedý *et al.* 2007, 2009a) abolished NPE development in Wistar rats subjected to rapid SCC.

Chronic hypertension is usually accompanied by a substantial reduction of baroreflex sensitivity as it was demonstrated in spontaneously hypertensive rats (SHR) (Andresen *et al.* 1980, Struyker-Boudier *et al.* 1982) and salt hypertensive Dahl rats (Miyjima and Bunag 1987, Andresen 1989, Brown *et al.* 1989, Nedvídek and Zicha 2000). The aim of the present study was to evaluate whether hypertension-associated impairment of baroreflex sensitivity might attenuate reflex bradycardia following SCC and thus exert some protection against NPE development in hypertensive animals.

Animals

We used 38 adult male rats (Institute of Physiology AS CR, Prague, Czech Republic) with body weights between 300-330 g. Animals were divided in the following groups: (1) Wistar rats (NPE model), (2) spontaneously hypertensive rats SHR, (3) salt-resistant Dahl rats fed a low-salt diet (DR-LS), (4) salt-resistant Dahl rats fed a high-salt diet (DR-HS), (5) salt-sensitive Dahl rats fed a low-salt diet (DS-LS), (6) salt-sensitive Dahl rats fed a high-salt diet for 4 weeks in adulthood (DS-HS). The low-salt diet contained 0.3 % NaCl, whereas there was 8 % NaCl in the high-salt diet. Animals were anesthetized with 1.5 % isoflurane in air (flow 300 ml/min) and arterial catheter for the monitoring of blood pressure and heart rate was inserted and exteriorized in the interscapular region. The animal was put in a prone position and the balloon compression of spinal cord was performed. Animals were sacrificed 10 min after lesioning, and the grade of neurogenic pulmonary edema was evaluated using macroscopic visual examination of subpleural bleeding and p-index (lung weight/body weight). Controls were healthy non-injured Wistar rats, sacrificed immediately after the induction of anesthesia. The possible role of isoflurane to induce neurogenic pulmonary edema *per se* was excluded in our previous study (Šedý *et al.* 2007). 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 Ethics Committee of the Institute of Physiology AS CR, Prague, Czech Republic.

Balloon-induced spinal cord injury

After the induction of anesthesia with 5 % isoflurane in room air (flow 300 ml/min), animals were maintained in 1.5 % isoflurane anesthesia (flow 300 ml/min) *via* a face mask throughout the operation. This concentration of isoflurane was shown to promote the severe neurogenic pulmonary edema in normal Wistar rats subjected to balloon-induced spinal cord injury (Šedý *et al*. 2007). For spinal cord injury, we used the model of an epidural balloon compression lesion, as described in detail previously (Vanický *et al.* 2001). Briefly, under aseptic conditions, a 2 cm median skin incision at the Th10-L1 level was made. 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 Fogarthy catheter (Baxter Healthcare Corporation, Irvine, CA, USA) was filled with distilled water and connected to a 50-µl Hamilton syringe and inserted into the dorsal epidural space 10 mm rostrally, to reach the Th8-Th9 spinal level. The balloon was rapidly inflated with 15 µl of distilled water for 5 min, using a micromanipulator. Subsequently, the balloon was deflated and removed.

Mean arterial pressure (MAP) and heart rate (HR)

were monitored in all animals using a PowerLab system (AD Instruments, Colorado Springs, USA). MAP (mm Hg) and HR (bpm) were monitored for 5 min before the procedure, throughout the entire procedure and for 10 min after the procedure. Baroreflex sensitivity was estimated from the changes of pulse interval (PI=60/HR) divided by MAP changes observed following SCC.

Evaluation of neurogenic pulmonary edema

The lungs were immediately removed from sacrificed animals and weighed. 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 (Šedý *et al.* 2007). Each lung was evaluated separately. 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 x 100), which has been previously considered to be very sensitive to the degree of pulmonary edema (Šedý *et al.* 2007).

Statistical analysis

The pulmonary index values, mean arterial pressure values and heart rate values are reported as mean

Table 1. The impairment of lung function after spinal cord injury in Wistar rats and SHR as well as in Dahl rats anesthetized by 1.5 % isoflurane.

Group	N	Absent (% of 2N)	Grade I (% of 2N)	Grade II (% of 2N)	Grade III (% of 2N)	Pulmonary index	Died (%)
Wistar	9	-	-	1 (6 %)	17 (94 %)	0.77 ± 0.05*	3 (33 %)
SHR	8	-	4 (25 %)	4 (25 %)	8 (50 %)	0.73 ± 0.05*	-
DR-LS	4	2 (25 %)	2 (25 %)	4 (50 %)	-	0.56 ± 0.05*	-
DR-HS	4	-	2 (25 %)	6 (75 %)	-	0.62 ± 0.03*	-
DS-LS	5	-	2 (20 %)	6 (60 %)	2 (20 %)	0.69 ± 0.05*	-
DS-HS	8	-	4 (25 %)	6 (38 %)	6 (38 %)	0.66 ± 0.03*	-
Intact controls	13	26 (100 %)	-	-	-	0.45 ± 0.02	-

The absence or presence of subpleural bleeding (evaluated as Grade I-III) in different groups is given as the total number of lungs in each group (the right and left lung were considered separately) and the percentage of all lungs examined in the respective group. A significant elevation ($p<0.05$) of the pulmonary index (mean values ± S.E.M.) in comparison with controls is indicated by an asterisk. Control animals are Wistar rats without spinal cord injury, sacrificed immediately after the onset of anesthesia. N – number of rats, 2N – number of lungs.

± S.E.M. The statistical significance between groups was compared using the non-paired Student´s t-test, the differences within the groups by the paired Student´s t-test.

Neurogenic pulmonary edema in Wistar rats and SHR

Balloon inflation in the spinal channel of Wistar rats caused considerable blood pressure elevation, heart rate decrease and severe neurogenic pulmonary edema in all cases. Moreover, 33 % of animals died due to pulmonary edema (Tables 1 and 2). SHR, which were characterized by higher MAP even under 1.5 % isoflurane anesthesia, responded to SCC with a comparable MAP increase and slightly enhanced HR reduction (Table 2). We did not observe impaired baroreflex sensitivity in SHR (3.74±1.05 vs. 2.42±0.72 ms/mm Hg in Wistar). Although no death occurred in SHR group, the degree of NPE (indicated by pulmonary index and the grade of subpleural bleeding) was not significantly attenuated as compared to normotensive Wistar rats (Table 1).

Neurogenic pulmonary edema in Dahl rats

High salt intake elicited hypertension in DS-HS rats which was comparable to that seen in SHR. Salt-resistant rats were characterized by higher baroreflex sensitivity compared to salt-sensitive ones (ΔHR/ΔMAP: DR-LS 5.34±1.33, DR-HS 5.79±1.82 vs. DS-LS 2.82±0.81, DS-HS 1.24±0.26 ms/mm Hg) because their HR reduction was relatively greater compared to salt-sensitive animals (Table 2). However, SCC elicited NPE in all groups of Dahl rats irrespective of the genotype or salt intake (Table 1).

Our experiments indicate that spinal cord compression induces neurogenic pulmonary edema even in rats with genetic or salt hypertension (SHR, salt-sensitive Dahl rats). Moderate attenuation of baroreflex sensitivity, which we have confirmed in salt hypertensive Dahl rats anesthetized with 1.5 % isoflurane, had no significant protective effects against NPE development. In fact, acute spinal cord compression produced considerable reduction of heart rate in both hypertensive groups (SHR –110 bpm; DS-HS –65 bpm). It should be noted that SCC did not induce any bradycardia in Wistar rats subjected to 3 % isoflurane anesthesia or atropine pretreatment (Šedý *et al*. 2009). In both cases a pronounced blood pressure rise after acute SCC did not elicit significant heart rate reduction.

It can be concluded that neurogenic pulmonary edema induced by acute spinal cord compression can occur even in hypertensive rats because their impairment of baroreflex sensitivity does not preclude significant bradycardia which is an essential prerequisite for this form of neurogenic pulmonary edema.

Table 2. Mean arterial pressure and heart rate: baseline values as well as the values found after particular surgical procedures and balloon compression in Wistar rats and SHR as well as in Dahl rats anesthetized by 1.5 % isoflurane.

Mean arterial pressure (mm Hg)						
	Wistar	**SHR**	**DR-LS**	**DR-HS**	**DS-LS**	**DS-HS**
Baseline values	101 ± 2	165 ± 3	99 ± 8	115 ± 1	117 ± 3	150 ± 6
Skin incision	89 ± 4*	118 ± 6*	96 ± 4	103 ± 2*	107 ± 4*	127 ± 7*
	(-12%)	(-28%)	(-3%)	(-10%)	(-9%)	(-15%)
Muscle incision	59 ± 4*	108 ± 6*	70 ± 7*	87 ± 1*	94 ± 8*	89 ± 8*
	(-42%)	(-35%)	(-29%)	(-24%)	(-20%)	(-31%)
Balloon insertion	109 ± 8	153 ± 11	98 ± 4	112 ± 4	124 ± 6	139 ± 7
	(+8%)	(-7%)	(-1%)	(-3%)	(+6%)	(-7%)
Balloon inflation – max	162 ± 4*	203 ± 5*	140 ± 5*	151 ± 6*	175 ± 3*	196 ± 4*
	(+60%)	(+23%)	(+41%)	(+31%)	(+50%)	(+31%)
Balloon inflation – 2 min	135 ± 5*	188 ± 4*	111 ± 6	126 ± 3*	139 ± 5*	157 ± 6
	(+34%)	(+14%)	(+12%)	(+10%)	(+19%)	(+5%)
Recovery	83 ± 6*	134 ± 4*	87 ± 5	97 ± 2*	99 ± 2*	100 ± 6*
	(-18%)	(-19%)	(-12%)	(-16%)	(-15%)	(-33%)
Heart rate (bpm)						
	Wistar	**SHR**	**DR-LS**	**DR-HS**	**DS-LS**	**DS-HS**
Baseline values	436 ± 16	358 ± 9	358 ± 17	360 ± 13	366 ± 12	366 ± 6
Skin incision	443 ± 9	326 ± 9	375 ± 17	394 ± 13	392 ± 17	373 ± 9
	(+2%)	(-9%)	(+5%)	(+9%)	(+7%)	(+2%)
Muscle incision	403 ± 9	302 ± 6*	373 ± 13	386 ± 11	374 ± 14	357 ± 22
	(-8%)	(-16%)	(+4%)	(+7%)	(+2%)	(-2%)
Balloon insertion	395 ± 12	295 ± 14*	334 ± 11	339 ± 16	311 ± 11*	289 ± 11*
	(-9%)	(-18%)	(-7%)	(-6%)	(-15%)	(-21%)
Balloon inflation – max	240 ± 19*	182 ± 18*	172 ± 39*	174 ± 24*	196 ± 10*	223 ± 16*
	(-41%)	(-49%)	(-52%)	(-52%)	(-46%)	(-39%)
Balloon inflation – 2 min	311 ± 21*	267 ± 13*	272 ± 15*	277 ± 8*	282 ± 13*	282 ± 15*
	(-29%)	(-25%)	(-24%)	(-23%)	(-23%)	(-23%)
Recovery	367 ± 15*	254 ± 10*	350 ± 20	386 ± 30	322 ± 7*	308 ± 14*
	(-16%)	(-29%)	(-2%)	(+7%)	(-22%)	(-16%)

Statistically significant (paired Student´s t-test, $p<0.05$) in-group differences in comparison to baseline values are marked with an asterisk. Relative changes from baseline values are shown in parentheses.

Conflict of Interest

There is no conflict of interest.

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