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Variations in Blood Pressure and Heart Rate in Conscious Rats with Cervical Lymphatic Blockade

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Abstract

The possible effects of cervical lymphatic blockade (CLB) on a series of parameters in conscious freely moving rats were analysed. Blood pressure (BP) and heart rate (HR) for conscious male Sprague-Dawley rats at 1, 3, 7, 11, 15 and 21 days after a CLB or a sham operation were monitored continuously for 24 hours with a computerized recording system. Since BP and HR were subjected to spontaneous variations, blood pressure variability (BPV) and heart rate variability (HRV) were expressed as the standard deviation of beat-to-beat BP and HR values. The baroreflex sensitivities (BRS) were determined by measuring the heart period (HP = 60,000/HR) prolongation in response to the elevation in BP induced by an intravenous administration of phenylephrine at 1, 7, 15 and 21 days after the CLB or sham operation. Compared with those in sham-operated rats, the values of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), HR and BRS in CLB rats were significantly lower, whereas the values of BPV and HRV were markedly raised in CLB rats at different time points. Furthermore, the impaired ultrastructure in the dorsomedial nucleus of the solitary tract (dmNTS) including degeneration, apoptosis and necrosis in neurons and gliacytes, were apparent from the 1st to 15th day but the changes were most significant at 7th day after CLB operation. Structural changes appeared to be closely related to functional changes of the dmNTS at each time point. Thus, in CLB conscious rats, a significant decline in blood pressure accompanied by dysfunction in its regulation might be due to the impaired structure in the dmNTS.

Key Words: cervical lymphatic blockade, blood pressure, heart rate, baroreflex sensitivity, conscious rats, ultrastructure, dorsomedial nucleus of the solitary tract

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Introduction

Many different types of brain damage, be it of a traumatic, infectious, metabolic or hypoxic origin, may result in cerebral dysfunction. In any case, maintaining basic hemodynamic and metabolic parameters at safe levels guarantees that cerebral perfusion pressure will remain at least at minimally acceptable levels, promoting the recovery from the pathophysiological procedure. The cervical lymphatic blockade (CLB)-induced alterations of many physiological indexes, such as cortex regional cerebral blood flow, somatosensory evoked potential, electroencephalogram and cervical lymphatic pressure, have been reported by this laboratory and others (24, 28, 29). However, the response of systemic arterial blood pressure to conditions of intracranial hypertension (ICH) and brain edema after CLB and the possible mechanism have not been systematically answered.

Consequently, the present work was designed to observe the variations of CLB on blood pressure (BP), heart rate (HR) and baroreflex function in conscious unrestrained rats with CLB using a computerized hemodynamic monitoring system. An attempt has also been made to interpret the mechanism involved in the above-mentioned variations by observing the ultrastructural changes in the dorsomedial nucleus of the solitary tract (dmNTS).

Materials and Methods

All experiments were approved by the local review committee and conducted in accordance with Animal Research Guidelines established by the Shandong University regulations on laboratory animal care.

Experimental Animals and Protocol

Male Sprague-Dawley rats at the age of 2 to 3 months, (250 g to 300 g) were purchased from the Experimental Animals Center of Shandong University (Jinan, Shandong, PRC). They were housed at controlled temperatures (23°C-25°C) and 8:00-20:00 light: dark cycle with free access to food and tap water. Animals were randomly divided into either sham or CLB group and each group was assigned to six time points: 1, 3, 7, 11, 15 and 21 days (n = 8 for each observation per time point). These rats were anesthetized with a mixture of ketamine (50 mg/kg) and diazepam (5 mg/kg) administered intraperitoneally. After acclimatization for more than 1 week, all rats were performed with a cervical lymphatic drainage blockade or a sham operation. Subsequently, these rats were intubated in the femoral artery and vein at -1, 1, 5, 9, 13 and 19 days after CLB or sham operation, respectively. Once the animals were conscious for 24 h from intubation operation under anesthetics (1, 21, 22), blood pressure monitoring system was connected. After approximately 24 h of habituation to BP recording environment, the BP signals were recorded continuously for 24 h in conscious, freely moving rats as postoperative hemodynamic parameters for 1, 3, 7, 11, 15 and 21 days.

Induction of CLB Model

Based on the method of Casley-Smith and Foldi (2, 8), the CLB model was performed with a slight modification of our previous studies (16, 18, 23, 29). These rats were anesthetized with a mixture of ketamine (50 mg/kg) and diazepam (5 mg/kg) administered intraperitoneally. To prevent respiratory congestion, atropine sulfate (0.4 mg/kg) was administered 10 mins prior to anesthesia. Briefly, the rats were fixed on the operation table, followed by median incision of the skin in the anterior region of neck. The fascia was then blunt dissected until the cervical lymph nodes, including bilateral submandibular superficial and deep nodes were identified and isolated under a dissecting microscope. The cervical lymphatic nodes were removed after obstructing their input and output tubes. A sham operation was performed in a similar manner but without the ligation of lymph tubes and the excision of the lymph nodes.

Measurement of Blood Pressure and Its Variability

At -1, 1, 5, 9, 13 and 19 days after a CLB or a sham operation, the rats were anesthetized again as described above. A floating polyethylene catheter was chronically placed into the lower abdominal aorta *via* the left femoral artery for the measurement of BP and HR, while another catheter was placed into the left femoral vein for intravenous injection. After 24 hours of recovery period from operation, systolic BP (SBP), diastolic BP (DBP), and HR were measured continuously using a computerized system (MPA 2000M, Alcott Biotech., Shanghai, PRC) described by Miao *et al*. (22). In short, just after catheterization, the rats were placed in individual cylindrical cages with free food and water. The aortic catheter was connected to a BP transducer (PT14M2, Department of Electronic Engineering, Fudan University, Shanghai, PRC) *via* a rotating swivel and the animals were allowed to move freely in their cages. After 24 h of habituation, the BP signal was digitized and beat-to-beat SBP, DBP, mean arterial blood pressure (MAP) and HR values were determined online. The mean values of SBP, DBP, MAP and HR during 24 h were calculated off line and served as SBP, DBP, MAP, and HR, respectively. The standard deviations of all values obtained during 24 h were calculated and denoted as the quantitative parameters of variability, *i.e.*, systolic blood pressure variability (SBPV), diastolic blood pressure variability

Table 1. Changes in BP and HR in rats with CLB.

Groups	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (bpm)
Normal	145.22 ± 7.33	104.05 ± 5.98	117.78 ± 6.43	385.65 ± 12.13
Sham				
1st day	142.26 ± 7.68	101.32 ± 5.68	114.97 ± 6.32	388.56 ± 13.31
3rd day	144.75 ± 7.28	103.33 ± 5.32	117.14 ± 5.97	385.32 ± 13.00
7th day	141.13 ± 7.53	99.85 ± 5.34	113.61 ± 5.97	390.36 ± 14.52
11th day	145.76 ± 7.26	103.95 ± 6.19	117.89 ± 6.40	387.82 ± 12.45
15th day	141.41 ± 7.60	100.70 ± 4.41	114.27 ± 5.47	385.71 ± 13.07
21st day	143.59 ± 7.37	102.56 ± 4.99	116.24 ± 5.78	386.07 ± 12.66
CLB				
1st day	133.88 ± 8.64*	94.87 ± 7.18*	107.87 ± 6.05*	379.02 ± 13.29*
3rd day	120.59 ± 9.14**^{b}	80.55 ± 7.47**^{b}	93.90 ± 7.29**^{b}	346.78 ± 15.67**^{b}
7th day	111.10 ± 10.79**^{bc}	70.46 ± 9.98**^{bd}	84.00 ± 7.56**^{bd}	324.06 ± 18.06**^{bd}
11th day	119.86 ± 9.18**^{be}	81.60 ± 7.79**^{bf}	94.35 ± 7.00**^{bf}	340.11 ± 16.09**^{be}
15th day	127.49 ± 8.25*^{cfg}	89.25 ± 6.57*^{dfg}	102.00 ± 6.81*^{dfg}	369.25 ± 15.99*^{dfh}
21st day	139.89 ± 7.05^{adfhj}	100.44 ± 5.95^{adfhj}	113.59 ± 6.32^{adfhj}	386.33 ± 12.69^{adfhj}

Data are expressed as mean ± SD (n = 8). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate. *P < 0.05, **P < 0.01, *vs*. Sham group; a P < 0.05, b P < 0.01, *vs*. 1st day of CLB group; c P < 0.05, d P < 0.01, *vs*. 3rd day of CLB group; e P < 0.05, f P < 0.01, *vs*. 7th day of CLB group; g P < 0.05, h P < 0.01, *vs*. 11th day of CLB group; i P < 0.05, j P < 0.01, *vs*. 15th day of CLB group.

(DBPV), and heart rate variability (HRV) (1, 21, 26), respectively.

Evaluation of Baroreflex Sensitivity (BRS)

BRS was estimated by the method of Smyth *et al*. (25), widely used for the evaluation of the regulation of cardiovascular activities. It allows measurement of the prolongation of heart period in response to an elevated BP. A bolus intravenous injection of phenylephrine was utilized and the dose was adjusted to the increase of SBP between 20 and 40 mmHg. The heart period (HP = 60,000/HR) (msec) was plotted against SBP for linear regression analysis and the slope of the SBP/HP relationship was expressed as BRS (msec/mmHg).

Electron Microscopic Studies on the dmNTS

After BP recording and BRS studies, 18 rats from each group (n = 3 for each time point) were anaesthetized and fixed for intracardiac perfusion. The rats were perfused through the ascending aorta with normal saline (0.9%) and 2.5% glutaraldehyde (4°C, pH 7.4) in 0.1 M phosphate buffer for 30 min and the brain stem was removed. Coronal brain sections (200 mm thick) of the dorsomedial part of the medulla oblongata, at the level of NTS (1 mm rostral to 1 mm caudal from the obex) (3), were postfixed with 2.5% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) for 2 h. Sections were soaked in 1% osmium tetroxide in 0.1 M cacodylated buffers for 2 h, rinsed in distilled water, followed by overnight staining with 1% aqueous uranyl acetate. Tissue sections were dehydrated in ascending series of ethanol to 100% followed by dry acetone, and embedded in epoxy resin. The localization of the dorsomedial subnucleus of nucleus of the solitary tract in ultrathin sections were confirmed and stained with uranyl acetate and counterstained with lead citrate and examined under JEM-1200EX electron microscope (JEOL Ltd., Tokyo, Japan).

Statistical Analysis

The values are expressed as mean ± S.D. The statistical significance of differences between the mean values of groups was first determined by using one-way ANOVA and then modified *t*-test was followed. Differences with a value of P < 0.05 were considered significant.

Results

Changes in BP and HR in Rats with CLB

Table 1 showed that the values of SBP, DBP, MAP

Table 2. Changes in BPV and HRV in rats with CLB.

Groups	SBPV (mmHg)	DBPV (mmHg)	HRV (bpm)
Normal	7.89 ± 0.78	6.60 ± 0.67	12.87 ± 1.71
Sham			
1st day	7.53 ± 0.66	6.85 ± 0.85	11.03 ± 1.50
3rd day	8.00 ± 0.79	7.05 ± 0.61	12.16 ± 1.45
7th day	7.75 ± 0.69	6.99 ± 0.60	11.94 ± 1.67
11th day	7.55 ± 0.68	7.03 ± 0.61	10.98 ± 1.23
15th day	7.98 ± 0.72	7.10 ± 0.64	12.23 ± 1.44
21st day	8.15 ± 0.81	7.01 ± 0.60	11.77 ± 1.66
CLB			
1st day	10.05 ± 0.81**	9.60 ± 0.66**	14.03 ± 1.04**
3rd day	11.04 ± 0.95**^{a}	10.54 ± 0.76**^{b}	15.62 ± 1.16**^{a}
7th day	12.14 ± 1.09**^{bc}	11.82 ± 0.77**^{bd}	16.83 ± 1.39**^{bc}
11th day	11.45 ± 1.08**^{b}	10.86 ± 0.62**^{bf}	15.70 ± 1.01**^{e}
15th day	10.00 ± 0.94*^{cfh}	9.84 ± 0.69**^{cfh}	14.12 ± 1.04**^{cfg}
21st day	8.25 ± 0.80^{bdfhj}	7.14 ± 0.61^{bdfhj}	12.25 ± 1.37^{bdfhi}

Data are expressed as mean ± SD (n = 8). SBPV, systolic blood pressure variability; DBPV, diastolic blood pressure variability; HRV, heart rate variability. *P < 0.05, **P < 0.01, *vs*. Sham group; a P < 0.05, b P < 0.01, *vs*. 1st day of CLB group; c P < 0.05, d P < 0.01, *vs*. 3rd day of CLB group; e P < 0.05, f P < 0.01, *vs*. 7th day of CLB group; g P < 0.05, h P < 0.01, *vs*. 11th day of CLB group; i P < 0.05, j P < 0.01, *vs*. 15th day of CLB group.

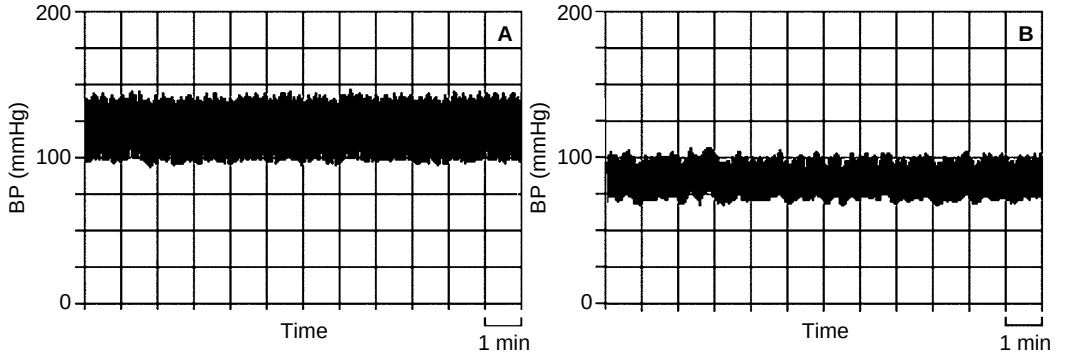

Fig. 1. Effects of CLB on BP in conscious rats.

and HR were not significantly different between normal and sham-operated rats (P > 0.05). However, the values at different time periods of the rats in CLB group were different from those of normal group from Day 1 to Day 15. For instance, the SBP, DBP, MAP and HR appeared to decrease significantly at day 1 after CLB operation (P < 0.05 *vs*. sham-operated group), and their valley values occurred at the 7th day in CLB group (111.10 ±10.79 mmHg, 70.46 ±9.98 mmHg, 84.00 ±7.56 mmHg and 324.06 ±18.06 bpm, respectively, P < 0.01 *vs*. sham-operated group) (Fig. 1B), then gradually increased after the 7th day in CLB rats, and finally returned to the base line at the 21st day in CLB group.

Changes in BPV and HRV in Rats with CLB

The mean values and standard deviations of BPV and HRV of different groups were presented in Table 2. SBPV, DBPV and HRV showed no obvious changes between the normal and sham-operated groups, but were changed in the CLB group. After CLB operation, the values of SBPV, DBPV and HRV were immediately increased from the 1st day (P < 0.01) onwards and the peak values occurred at the 7th day with the increase of 56.65%, 69.10% and 40.95%, respectively, compared with those in sham-operated groups (P < 0.01). These augmentations of SBPV, DBPV and HRV were gradually decreased after the peak at the 7th day, and finally returned to the base line level at the 21st day in CLB rats (P > 0.05 compared with the control).

Changes in BRS in Rats with CLB

In physiological conditions, the stability of BP

Table 3. Changes in BRS in rats with CLB (ms/mmHg).

Groups	1st day	7th day	15th day	21st day
Normal	1.06 ±0.08	–	–	–
Sham	1.05 ±0.10	1.00 ±0.09	1.02 ±0.10	1.04 ±0.07
CLB	0.80 ±0.08**	0.70 ±0.06** [a]	0.78 ±0.07** [b]	1.05 ±0.10 [ace]

Data are expressed as mean ± SD (n = 8). BRS, baroreflex sensitivity. **$P < 0.01$, *vs.* Sham group; a $P < 0.01$, *vs.* 1st day of CLB group; $P < 0.05$, c $P < 0.01$, *vs.* 7th day of CLB group; $P < 0.01$, *vs.* 15th day of CLB group.

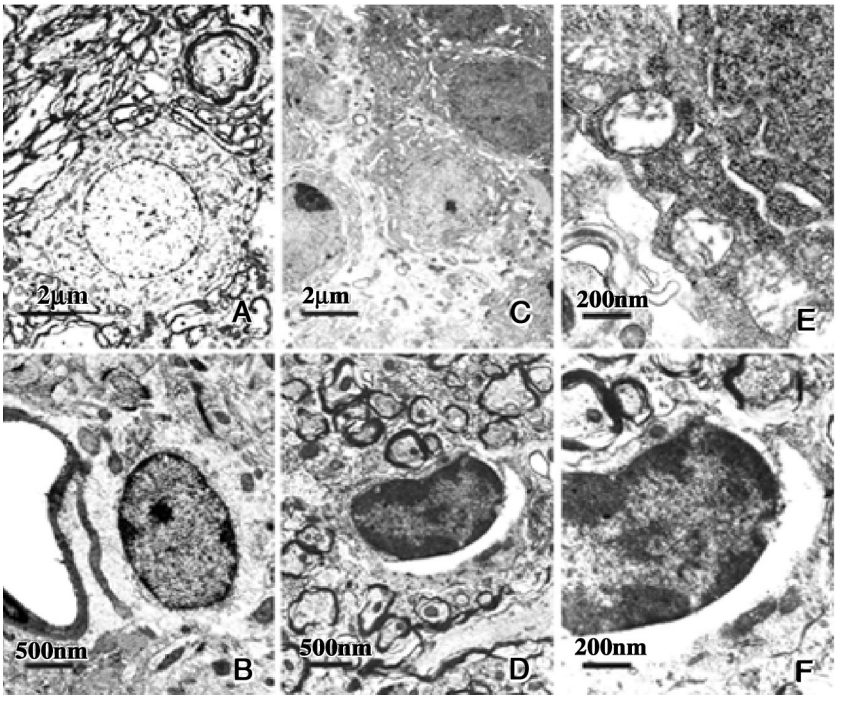

Fig. 2. Ultrastructural changes in damaged cells in the dorsomedial nucleus of the solitary tract in CLB rats.

is maintained by arterial baroreflex (ABR), which acts as an effective buffer for BP fluctuations. To evaluate the function of ABR in conscious rats, the mean values of BRS were measured at 1, 7, 15 and 21 days and depicted in Table 3. No statistical difference among the values of BRS at the different time points during the experiment was found in sham groups, and there was no obvious change in the values of BRS between normal and sham-operated groups. On the contrary, BRS significantly decreased at different time points in the CLB group compared with those in the sham-operated group ($P < 0.01$) with the decreases of 23.81%, 30% and 23.53% at 1, 7 and 15 days, respectively. Moreover, the valley value appeared on the 7th day, and returned to the normal level on the 21st day after CLB operation.

Electron Microscopic Changes in the dmNTS

To investigate the mechanism involved in the alternations of the parameters mentioned above, observation on ultrastructural changes in the dmNTS was performed. Under the transmission electronic microscopy, the neurons, gliacytes, microvascular endotheliocytes as well as basement membrane in sham-operated tissues were well preserved. Normal, homogenously distributed nuclear chromatin, distinct mitochondria cristae, double-lined nuclear and perfect membrane system are also clearly seen (Fig. 2, A and B).

In contrast to those of the sham-operated rats, the morphological changes in the dmNTS appeared from the 1st to 15th day after CLB operation. The neurons exhibited a significant degeneration, including cell shrinkage, vacuoles or cavities alternation with pyknotic, asymmetrical nuclei, hyperchromatin and clumps of heterochromatin around the edge of the nuclear membrane (Fig. 2, C and D). In addition, the whole membrane system in neurons including endoplasmic reticulum, mitochondrial and nuclear

membrane was damaged, notably mitochondria cristae were swollen, enlarged, turned lucent and disarrayed, fragmented and finally disappeared (Fig. 2E). In the gliocytes, the membrane edema occurred with periphery vacuolar degeneration, nuclear shrinkage, chromatin condensation, and void space was evident (Fig. 2, D and F). Edematous fluid accumulated around microvessels, leading to the dilated and congested microvascular endothelium with partial breaks. All above morphological alterations were most prominent on the 7th day in CLB rats.

Discussion

In brain, cervical lymphatic vessels play an important role in maintaining homeostasis of the internal environment and regulating intracranial pressure (ICP) for its normal physiological functions (7, 9, 20). Studies have demonstrated that lymphatic drainage is involved not only in circulating cerebrospinal fluid (CSF) (12, 13, 24, 31), but also in the cleaning-up of macromolecules in the CSF, the interstitial space of the brain and the brain parenchyma (15,17). We believed that blood-brain barrier (BBB) may be damaged severely under CLB, whilst substantial amounts of plasma proteins possibly penetrate into interstitial spaces (23, 27). Therefore, surgical blockade of the cervical lymph drainage is expected to induce an increase in protein content and colloid osmotic pressure of interstitial fluid in the brain, so various micromolecules penetrate into the brain tissue interspaces thereby aggravating interstitial edema, leading to ICH and lymph-stagnation cerebral edema, and inducting a series of changes of cerebral function (7, 12, 13).

The impact of CLB on cardiovascular function in anaesthetized rats is well documented (6, 28); however, for the first time the present study describes the measurement of hemodynamic parameters in unrestrained conscious CLB rats within 48 hours of recovery from anesthetic conditions. Therefore, it may mimic the clinical situation that patients suffered from lymphatic lesions. It appeared that BP in conscious freely moving rats was significantly lowered from day 1 after CLB, reaching a valley value on the 7th day, and recovered to baseline on the 21st day (Table 1). On the contrary, the blood pressure in anaesthetized CLB rats increased at 2 and 3 week after CLB operation (28). The contradictory result verifies that BP and baroreflex function are largely inhibited by anaesthesia (30). It is not surprising that the magnitude of reduction in BRS (only 0. 07 ± 0. 01 ms/mmHg) in anaesthetized rats (6), is far below the values that were measured in conscious CLB rats as provided in the present by the computerized hemodynamic monitoring system.

Generally speaking, in the physiological system BP and HR are not constant but undergo spontaneous variations. However, their stabilities are regulated *via* ABR that acts as an effective buffer and prevents excessive BP and HR swing (14). In some studies, BPV has been expressed as the standard deviation and/or coefficient of variation (19, 26), which reflects the quality and degree with which BP fluctuates within a permissible range. And the similar situation is suitable for HRV. Though BPV and HRV are related to many factors, of which arterial baroreceptor reflex is vital to sustain arterial blood pressure and heart rate steady. The complicated regulatory circuit processes and integrates the afferent message of BP and HR alternations from periphery, followed by fulfilling the regulatory function on BP and HR by altering the activity of autonomic nerve. As a result of a series of neural projections, decreased sympathetic nerve activity in CLB rats would reduce the BP and increase the BPV. Diminished HR after CLB might be primarily due to an enhancement of activity of the vagal efferents to the heart. In addition, the decrease of sympathetic outputs after CLB might have also facilitated the descent in HR.

The present study demonstrated that the levels of BP, BPV and BRS changed notably during 24 h in CLB group. In other words, CLB operated rats were less sensitive and showed reduced cardiovascular responses to environmental stimuli compared with sham operated rats. This is probably because to that CLB operation brings edema in whole brain, including the NTS. It is clear that the NTS plays a key role in cardiovascular regulation, because baroreceptor and chemoreceptor afferent fibres terminate in the location (4), particularly the dmNTS is preferentially barosensitive (10). Here, the images from transmission electronic microscope demonstrated that the neurons and gliocytes in the dmNTS showed a significant degeneration, apoptosis and necrosis, including cells shrunken, vacuoles or cavities alternating with pyknotic, asymmetrical nuclei and clumps of heterochromatin around the edge of the nuclear membrane, and so on (Fig. 2). Ultrastructural changes in the dmNTS appeared to be closely related to changes in BP, BPV and BRS at each time period. Thus, a significant decline in BP accompanied by dysfunction in its regulation seen in CLB conscious rats may be due to the impairment of the dmNTS.

Our earlier study showed that cerebral blood flow in cortical region was remarkably decreased after the 1st, 5th and 7th days of CLB operation (29) and consistent with the reduction of systemic BP after CLB. Therefore, hypotension induced by CLB may play a neuroprotective role for the ICH (ICH has to be defined) and prevent brain edema. The decrease in systemic BP may reduce the pressure on the cerebral microcirculation, and diminish cerebral perfusion

pressure to minimize the formation of edema and relieve ICH (5). Subsequently, *i.e.*, after 7 days of CLB, accompanied by the alleviation of the structure in the dmNTS, the function of ABR was ameliorated and BP and HR were raised step by step. However, the exact pathway needs further study by which systemic arterial pressure can augment the extracranial transport of CSF into cervical lymph nodes.

Putting all the results together, our data suggest that CLB may provoke the alterations and variations of BP and ABR in conscious rats, and damage the dorsomedial nucleus of the solitary tract. Furthermore, the functional changes are in tight concordance with the structural changes in NTS at the given time points investigated in CLB rats. It is concluded that brain edema and ICH after CLB may evoke cardiovascular dysfunction in conscious rats, which may originate from the impaired structure in the dmNTS by CLB. The precise correlation of cerebral lymphatic microcirculation with systemic circulation requires further study.

Acknowledgments

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