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Positive End-Expiratory Pressure Depressed Cardiovascular Autonomic Nervous System Activity in Acute Brain Damaged Rabbits under General Anesthesia

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ABSTRACT

Artificial ventilation with positive end-expiratory pressure (PEEP) is commonly applied for brain damaged patients. However, the effect of the ventilation on brain function, including cardiovascular autonomic nervous system (CANS) activity, is not well elucidated. In order to investigate the effect of 5 cmH_2O PEEP on CANS activity in brain damaged rabbits under general anesthesia, we produced acute brain damage by intracranial balloon inflation. Measurements were made before (control) and after application of PEEP, and after inflation with incremental volume of the balloon. Power spectral analyses of heart rate variability (HRV) and systolic arterial pressure variability (SAPV) were used for the assessment of CANS activity. Spectral powers in the low-frequency range of 0.04 to 0.40 Hz (LF) and high-frequency range of 0.75 to 1.40 Hz (HF) were computed, and their ratio LF/HF was assessed as the neural balance of CANS. The animals in group P were ventilated with 5 cmH_2O PEEP, while those in group Z were ventilated with zero end-expiratory pressure. Colored microsphere counting was used for the assessment of brain circulation. In the results, PEEP had no effect on HRV and SAPV parameters before induction of brain damage. After inflation with incremental volume of the balloon, log (HF) and log (LF) in group P were lower than in group Z in HRV analysis, and log (LF) in group P was lower than in group Z in SAPV analysis. Microsphere counting revealed that brain blood flow was reduced during the progression of brain damage and showed a significant difference after application of PEEP between the groups. We concluded that 5 cmH_2O PEEP depressed CANS activity during the progression of brain damage in rabbits and that this was partly due to aggravated brain function induced by PEEP.

Key words: *Heart rate variability, Positive end-expiratory pressure, Mechanical ventilation, Brain damage*

Brain damaged patients are often treated with artificial ventilation in critical care settings. Positive end-expiratory pressure (PEEP) is commonly applied for such critically ill patients, since PEEP can increase functional residual capacity and reduce intrapulmonary shunting to improve oxygenation. PEEP may also increase intracranial pressure (ICP) and decrease cerebral perfusion pressure (CPP), leading to aggravated neurological function[1] including cardiovascular autonomic nervous system (CANS) activity. In patients with normal ICP, it is reported that applying low level PEEP does not affect ICP or CPP, whereas high level PEEP significantly increases ICP[18]. It is also documented that PEEP has no effect on ICP or CPP in intracranial hypertensive patients, indicating the safety of low-level PEEP[18]. PEEP within 5 cmH_2O is thus recommended for respiratory care of brain dead patients[22]. However, the effect of the ventilation on brain function, including CANS activity, is not well elucidated in those with brain damage.

Heart rate variability (HRV) analysis, useful for representing the condition of the central nervous system including CANS[2], is applicable for clinical settings, since it is a non-invasive measuring tool to estimate the brain function. Systolic arterial pressure variability (SAPV) analysis is also used clinically and experimentally. CANS activity has been studied in chronic brain damage or brain death[3,27]; however, little is known about the relationship between CANS activity and acute brain damage[4,8]. We therefore investigated the effect of 5 cmH_2O PEEP on CANS activity during acute

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brain damage in rabbits by analyzing HRV and SAPV.

MATERIALS AND METHODS

Animal preparation

This study was approved by the Institutional Animal Care Committee of Hiroshima University. Sixteen adult Japanese white rabbits (weighing 2.7 to 3.4 kg) were used. The animals were allowed access to food and water *ad libitum* before the study, and all experiments were conducted in the morning. A continuous infusion of lactated Ringer's solution was started at 5 $ml \cdot kg^{-1} \cdot h^{-1}$. The animals were anesthetized with an intravenous injection of pentobarbital (30 $mg \cdot kg^{-1}$) via an auricular vein, positioned supinely, and then tracheostomized. A 3.5 mm ID tube was inserted midway along the trachea, after which controlled mechanical ventilation with a ventilator (Servo 900B, Siemens-Elema, Solna, Sweden) was initiated using zero end-expiratory pressure with a frequency of 60 cycles/min and an inspiratory-pause-expiratory ratio of 25:10:65. General anesthesia was maintained with end-expiratory isoflurane of 1% in humidified oxygen, with the fractional concentration of O_2 at 1.0. The right carotid artery and left internal jugular vein were cannulated with polyethylene catheters to monitor both arterial pressure and central venous pressure. Blood samplings were drawn after each measurement, and arterial blood gases and pH were determined (ABL4, Radiometer, Copenhagen, Denmark).

The minute ventilation volume was adjusted to achieve normocapnia with $PaCO_2$ between 35 and 45 mmHg. End-tidal CO_2 and isoflurane concentration were measured breath by breath with a gas monitor (MultiCap, Datex, Helsinki, Finland). Once the minute ventilation volume was adjusted, it was rigorously maintained by monitoring end-tidal CO_2 throughout the study. End-tidal CO_2 was determined to be around 10 mmHg below the $PaCO_2$ at the preliminary setting. Paralysis was achieved with a bolus injection of vecuronium (1.0 $mg \cdot kg^{-1}$), followed by a continuous infusion of 2.0 $mg \cdot kg^{-1} \cdot h^{-1}$. Chest wall lead electrocardiogram and heart rate (HR) were monitored. The animal was then turned prone and the head placed on a stereotactic apparatus. The scalp was incised along the midline and the skin flaps reflected. A parietal burr hole 5 mm in diameter was drilled into the left side of the skull for insertion of an epidural pressure bolt. The bolt was molded in place with cyanoacrylate cement for monitoring ICP. Another parietal burr hole 2 mm in diameter was drilled into the right side of the skull for insertion of a 4 Fr Fogarty catheter into the extradural space to increase ICP.

All pressure measurements were made using transducers (Uniflow, Baxter, Salt Lake, UT) and recorded with a polygraph (RM6000, Nihon Kohden, Tokyo, Japan). Zeroing of the pressure transducers was made at the level of the anterior axillary line for arterial pressure and central venous pressure monitoring, and at the external acoustic meatus for ICP monitoring. ICP was increased over a period of approximately 60 min. Increments of 0.25 ml of sterile water were added to the sealed extradural balloon every 15 min up to a total amount of 1.0 ml. A heating lamp was used to maintain body temperature, which was monitored with a rectal thermistor (MGA-III, Nihon Kohden).

The animals were randomly assigned into two groups. The rabbits in group P were ventilated with 5 cmH_2O PEEP and those in group Z were ventilated with zero end-expiratory pressure. Measurements were taken for 5 min during each of the following periods: before insertion of the intracranial balloon (M0), before application of PEEP for ventilation (M1: control), after application of PEEP (M2), 5 min after inflation with 0.25 ml of water (M3), 5 min after inflation with 0.5 ml of water (M4), 5 min after inflation with 0.75 ml of water (M5), 5 min after inflation with 1.0 ml of water (M6), and 20 min after inflation with 1.0 ml of water (M7).

To confirm brain activity, we measured the 90% spectral edge frequency (SEF90) by monitoring the electroencephalogram throughout the study. To quantify cerebral blood flow, 1×10^6 colored microspheres (Ultrasphere, E-Z Trac, Los Angels, CA) per injection were given over 10 sec with 2 ml of saline through the intra-aortic catheter at the time of M2, M4, and M7. Plasma catecholamine levels were also measured at the time of M2, M4, and M7. The subjects receiving general anesthesia with isoflurane were sacrificed by KCL injection at the end of the study, and the brain was immediately excised. The tissue was hydrolyzed to count the microspheres microscopically.

Data analysis

Computations were made using a personal computer with custom software that we developed. In brief, the recorded ECG was replayed off-line to digitize the samples at 2 kHz by using a low-cut filter (cut-off frequency: 0.01 Hz). R-R interval tachograms were made every 256 seconds in all animals. By sampling at 4 Hz, a 1024 point instantaneous HR was made from the tachograms and subjected to a fast Fourier transform in order to obtain the power spectra. Spectra less than 2.00 Hz were normalized by the square of the mean of the HR. Spectral accuracy was confirmed by testing a sequence of simulated R-R intervals generated by an integral pulse frequency modulation model.

Spectral power was calculated by integration of the spectral components of the total (TP: 0.04–2.00

Hz), low (LF: 0.04–0.40 Hz) and high (HF: 0.75–1.40 Hz) frequency band areas[13]. Integrated components and the LF/HF ratio are presented as a common logarithm. Systolic arterial pressure data corresponding to each HR was used for SAPV analysis in the same analytical manner as for the HRV analysis. LF and HF band areas in SAPV were determined the same as in HRV.

Statistical analysis

Data are presented as mean ± SD or median (range). Mann-Whitney's U test was used to compare non-parametric variables including, catecholamine levels, between the groups. Wilcoxon's signed-rank test was used for comparison within a group. Student's t-test was used to compare parametric variables including HRV and SAPV data between groups, and repeated-measure analysis of variance followed by a Dunnett test was used within a group. Statistical significance was considered when p was less than 0.05.

RESULTS

Serial changes of the measured parameters are listed in Table 1. Just after inflation of the intracranial balloon, ICP transiently increased and was restored during each measurement. Significant decreases were observed in CPP at M6. The mean value of SEF90 significantly decreased at M4, and became absent at M6, indicating brain death.

Serial changes in HRV parameters are shown in Fig. 1. Mean R-R intervals significantly increased at M5, M6 and M7 in group Z, and at M6 and M7 in group P. Log (LF) significantly increased at M3, M5 and M6 in group Z, and at M6 in group P. Log (HF) remained consistent in group Z; however, it decreased at M5, M6 and M7 in group P. Log(LF/HF) significantly increased at M5 in group Z, and at M5 and M6 in group P. Between groups, log (HF) at M6 and log (LF) at M3 in group P were significantly lower than those in group Z. The HRV parameters suggested that PEEP depressed both sympathetic and parasympathetic nervous system activity during the progression of brain damage.

Serial changes in SAPV parameters are shown in Fig. 2. SAP significantly decreased at M6 in both groups. Log (LF) significantly increased at M5 in group Z; however, it remained constant in group P. Log (HF) significantly decreased at M5 in group Z, while it decreased at M3, M4 and M5 in group P. Log (LF/HF) significantly increased at M5 in group Z; however, it remained constant in group P. Between groups, log (LF) was significantly lower at M5 in group P than in group Z. The SAPV parameters also suggested that PEEP depressed both sympathetic and parasympathetic nervous system activity during the progression of brain damage.

Blood gas data and plasma catecholamine levels are listed in Table 2. $PaCO_2$ increased at M7 in

Table 1. Variables during procedure

Group	M0	M1	M2	M3	M4	M5	M6	M7
Mean Arterial Pressure (mmHg)								
Group Z	95.4 ± 11.2	90.9 ± 12.9	90.9 ± 12.9	90.0 ± 9.9	91.4 ± 9.7	89.5 ± 19.3	53.0 ± 15.2*	42.0 ± 12.6*
Group P	94.5 ± 9.8	85.9 ± 10.2	84.8 ± 8.0	86.2 ± 10.0	91.0 ± 12.8	93.9 ± 12.8	55.6 ± 26.9*	44.8 ± 19.3*
Central Venous Pressure (mmHg)								
Group Z	6.0 ± 2.6	6.1 ± 2.1	6.1 ± 2.1	6.0 ± 2.1	5.8 ± 2.3	5.8 ± 2.3	6.3 ± 2.7	5.9 ± 2.9
Group P	3.3 ± 5.3	3.8 ± 5.4	4.1 ± 5.1	4.3 ± 5.4	4.3 ± 5.4	4.8 ± 5.4	4.3 ± 5.4	4.1 ± 5.5
Peak Airway Pressure (mmHg)								
Group Z	8.5 ± 1.4	8.6 ± 1.0	8.6 ± 1.0	8.7 ± 1.3	8.7 ± 1.3	8.6 ± 1.3	8.4 ± 1.0	8.4 ± 1.0
Group P	8.9 ± 0.9	9.5 ± 1.1	9.6 ± 1.3	9.8 ± 0.9	9.9 ± 1.3	10.0 ± 1.4	9.8 ± 1.3	9.8 ± 1.2
Intracranial Pressure during measurement (mmHg)								
Group Z		8.6 ± 1.2	8.6 ± 1.2	9.8 ± 1.8	10.7 ± 3.6	11.3 ± 5.8	6.9 ± 4.5*	7.5 ± 3.4
Group P		9.3 ± 5.8	8.7 ± 3.2	9.3 ± 3.4	13.4 ± 8.5	16.9 ± 13.3	16.0 ± 12.0	11.4 ± 9.6
Cerebral Perfusion Pressure during measurement (mmHg)								
Group Z	89.5 ± 11.2	82.1 ± 12.1	82.1 ± 12.1	80.0 ± 9.3	80.1 ± 8.7	77.9 ± 17.7	45.3 ± 15.0*	34.4 ± 10.5*
Group P	91.1 ± 11.0	74.9 ± 9.0	75.6 ± 7.9	75.1 ± 10.1	75.6 ± 13.4	71.3 ± 19.9	36.0 ± 29.0*	30.9 ± 20.1*
Spectral Edge Frequency 90 (Hz)								
Group Z	8.4 ± 2.0	9.1 ± 3.0	9.1 ± 3.0	7.6 ± 2.5	6.8 ± 2.4*	3.9 ± 2.5*	0.0*	0.0*
Group P	9.2 ± 2.5	9.1 ± 2.3	7.5 ± 1.9	8.0 ± 4.1	6.8 ± 3.0*	1.8 ± 3.3*	0.0*	0.0*

Variables were taken at 8 different measurements as follows: M0, before insertion of the intracranial balloon; M1, before application of PEEP for ventilation; M2, after application of PEEP in group P (in group Z, M1 = M2); M3, 5 min after inflation with 0.25 ml of water; M4, 5 min after inflation with 0.5 ml of water; M5, 5 min after inflation with 0.75 ml of water; M6, 5 min after inflation with 1.0 ml of water; M7, 20 min after inflation with 1.0 ml of water.
Data are presented as mean ± SD. *$p < 0.05$ vs. M1 within a group.

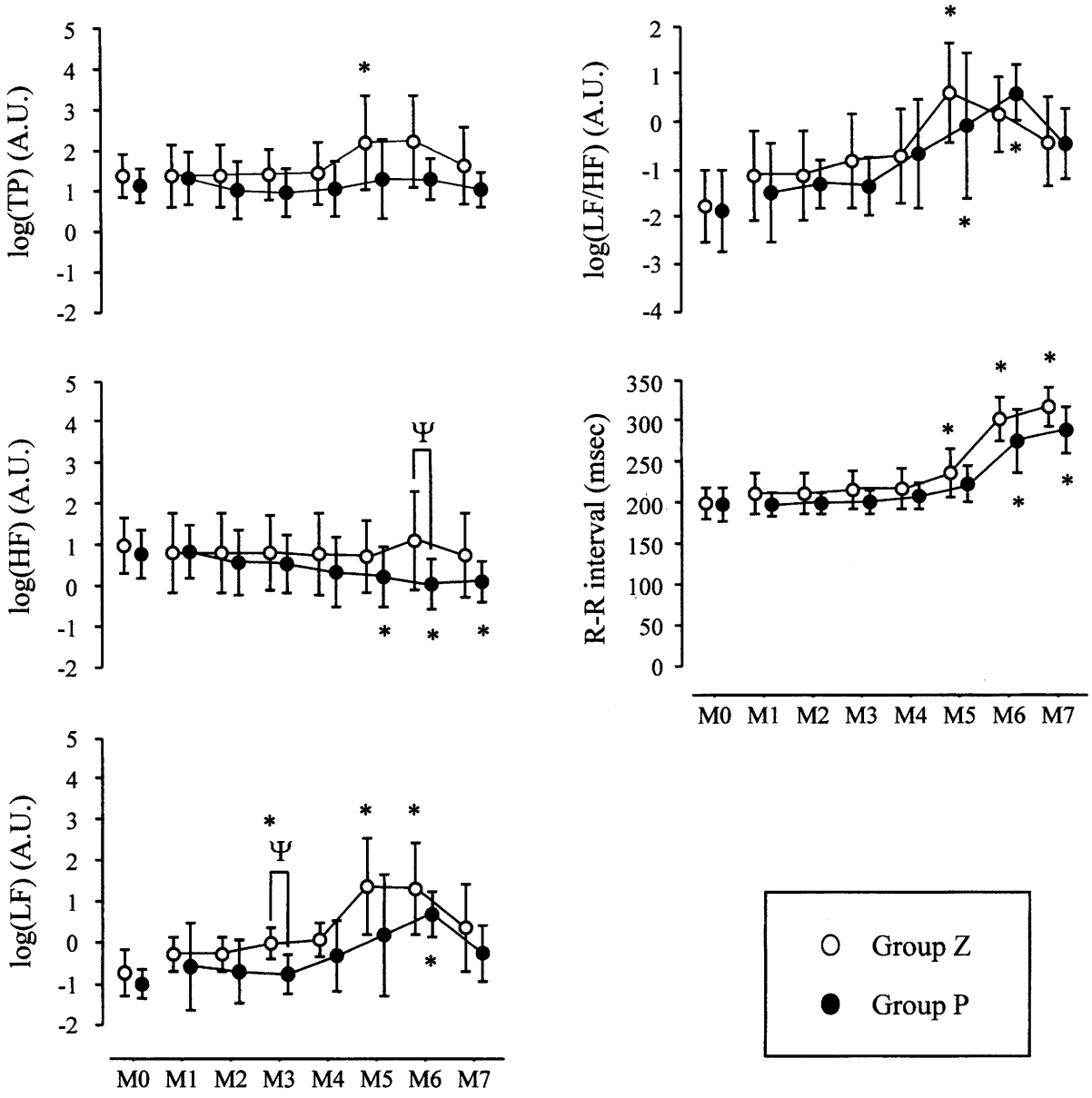

Fig. 1. Spectral components of HRV and R-R intervals during procedure.
Timings shown are same as in Table 1. Data are presented as mean ± SD.
*p < 0.05 vs. M1 in the same group.
ψp < 0.05 for intergroup difference at same timing.

Abbreviations are: TP, total power; HF, high frequency; LF, low frequency; AU, arbitrary unit.

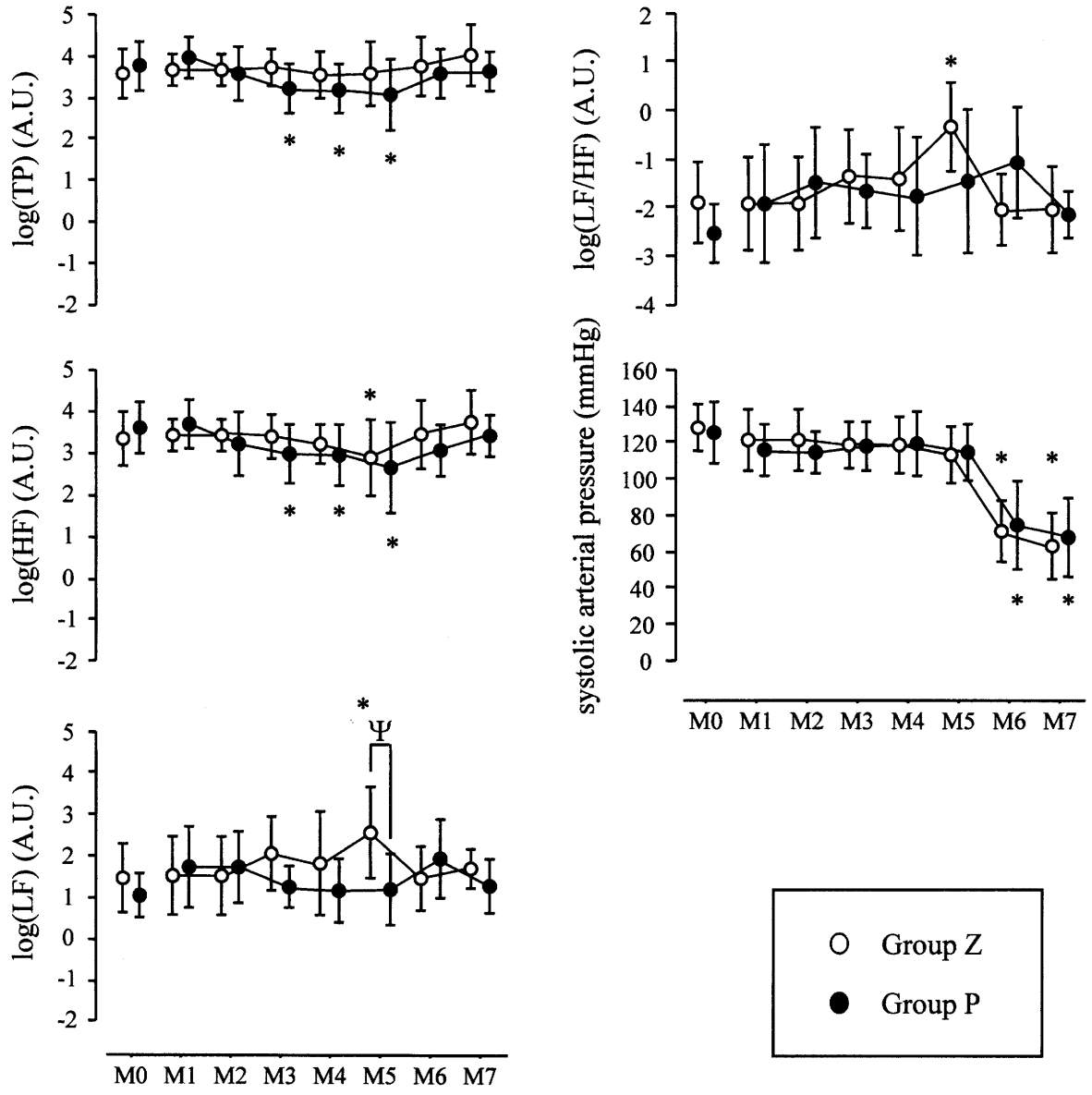

Fig. 2. Spectral components of SAPV and systolic arterial pressure during procedure.
Timings shown are same as in Table 1. Data are presented as mean ± SD.
*$p < 0.05$ vs. M1 in the same group.
ψ$p < 0.05$ for intergroup difference at same timing.

Abbreviations are: TP, total power; HF, high frequency; LF, low frequency; AU, arbitrary unit.

Table 2. Blood gas data and plasma catecholamine levels during procedure

Group	M1	M2	M4	M7
pH				
Group Z	7.40 ± 0.3	7.40 ± 0.3	7.41 ± 0.3	7.39 ± 0.3
Group P	7.42 ± 0.5	7.38 ± 0.6	7.39 ± 0.6	7.36 ± 0.6*
$PaCO_2$ (mmHg)				
Group Z	40.3 ± 2.8	40.3 ± 2.8	40.9 ± 3.4	42.0 ± 3.1
Group P	39.8 ± 5.0	40.7 ± 5.2	41.1 ± 4.3	43.4 ± 4.1*
PaO_2 (mmHg)				
Group Z	516.7 ± 28.0	516.7 ± 28.0	508.3 ± 26.5	506.6 ± 31.9
Group P	520.4 ± 23.6	501.0 ± 24.8	518.6 ± 13.0	494.5 ± 37.0
Epinephrine ($pg \cdot ml^{-1}$)				
Group Z		9 (9– 15)	10 (5– 23)	5 (5–42)
Group P		17 (6– 72)	33 (10–103)	7 (5–15)*#
Norepinephrine ($pg \cdot ml^{-1}$)				
Group Z		90 (35–509)	91 (34–477)	31 (9–58)*#
Group P		132 (46–387)	146 (56–371)	49 (15–75)*#ψ
Dopamine ($pg \cdot ml^{-1}$)				
Group Z		5 (5– 9)	< 5	< 5
Group P		< 5	< 5	5 (5– 8)

Values are means ± SD or median (range).
*p < 0.05 vs. M1 for blood gas data.
*p < 0.05 vs. M2, #p < 0.05 vs. M4 for catecholamine levels.
ψp < 0.05 for intergroup difference at same timing.
Timings shown are same as in Table 1.

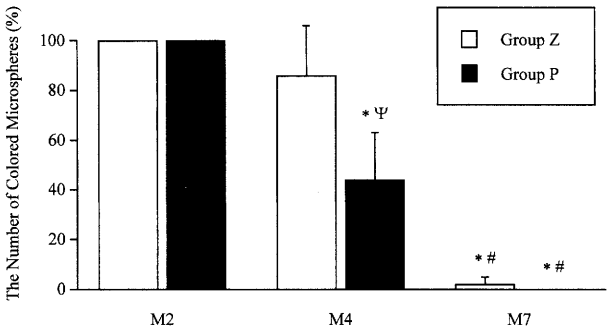

Fig. 3. Changes of the number of colored microspheres.
Timings shown are same as in Table 1. Values are means ± SD.
*p < 0.05 vs. M2 in the same group.
#p < 0.05 vs. M4 in the same group.
ψp < 0.05 for intergroup difference at same timing.

group P. The plasma norepinephrine level decreased at M7 in both groups, though the level in group P was significantly higher than that in group Z. The body temperature ranged from 37.2 to 38.8 °C. Serial changes in the number of colored microspheres counted are shown in Fig. 3. The number in group P was significantly lower at M4 than M2 and significantly lower than in group Z. No microspheres were detected at M7 in either group, suggesting that brain death was achieved.

DISCUSSION

After application of 5 cmH_2O PEEP, the number of colored microspheres in group P was significantly lower than in group Z. In addition, 5 cmH_2O PEEP had no effect on HRV and SAPV parameters before induction of brain damage; however, log (HF) at M6 and log (LF) at M3 in HRV and log (LF) at M5 in SAPV were significantly different between the groups. We therefore considered that 5 cmH_2O PEEP depressed CANS activity during the progression of brain damage and that 5 cmH_2O PEEP could aggravate brain function.

HRV and SAPV analyses are useful tools for investigating CANS activity since they are less invasive to apply in clinical conditions. Although the analyzed band width is different between human and animal, the interpretation of the meaning of LF and HF is not different. Namely, the LF component, which is related to cyclic fluctuations in peripheral vasomotor tone (Mayer wave at 0.3 Hz in rabbits) as well as the frequency response of the baroreceptor reflex, is mediated by a combination of sympathetic and parasympathetic activity. The HF component is associated with oscillations as a result of the respiratory cycle (respiratory sinus arrhythmia) mediated by the vagus[16]. Thus, the LF/HF ratio is assumed to be an index of sympatho-vagal balance[20].

Brain damage, frequently associated with the Cushing reaction, may develop hemodynamic deterioration with severe hypotension and bradycardia, because the sympathetic outflow may be abruptly

depressed. Herijgers et al[12] investigated brain death in rats by using an intracranial balloon inflation. In their study, LF in HRV did not change, while HF increased significantly 30 min after brain death. In the blood pressure variability analysis, LF decreased significantly 30 min after brain death. Kawamoto et al[14] investigated brain death in rabbits by using an intracranial balloon inflation. In their HRV analysis, both LF and HF were decreased significantly 60 min after brain death, though the decrease in HF was the more remarkable, suggesting the progression of the vagolytic condition. In many studies of chronic brain damage or brain dead subjects, LF and/or the LF/HF ratio decreased significantly[3,7,9,15,19,21,27]. In the present study, serial changes in the parameters of the HRV and SAPV analyses suggested that progressive brain damage caused by intracranial balloon inflation activated cardiovascular sympathetic activity, and that completed brain death is associated with depressed sympathetic tone. We considered that the abrupt hemodynamic deterioration observed in the present study was due to a decrease of afterload induced by a withdrawal of sympathetic tone.

In this study, SEF90 and colored microspheres were used as objective tools to estimate brain function. Absence of these parameters suggested that the brain function was completely demolished. The mean value of SEF90 significantly decreased at M4, and became absent at M6 showing brain death. Non-radioactive colored microspheres, as useful as radioactive ones, can well reflect regional organ blood flow[10]. In the present model, an insignificant decrease of the microspheres at M4 and their absence at M7 in group Z seemed to reflect the serially reduced brain circulation. We thus considered that brain death was achieved at M6 and M7.

It is controversial whether PEEP increases ICP and reduces CPP. McGuire et al[18] investigated neurosurgical patients receiving mechanical ventilation with 5, 10, and 15 cmH_2O PEEP. In patients with normal ICP, an application of 5 cmH_2O PEEP did not affect ICP and CPP, while both 10 and 15 cmH_2O PEEP increased ICP significantly. In intracranial hypertensive patients, all levels of PEEP had no effect on ICP and CPP, indicating that 5 cmH_2O PEEP was also safe. In the management of brain dead donors, PEEP within 5 cmH_2O is therefore recommended for respiratory care[22].

There are a few studies on the effect of PEEP on CANS activity during mechanical ventilation. Selldén et al[23-25] investigated rats and healthy volunteers undergoing mechanical ventilation with various levels of PEEP, during which they studied the effect of PEEP on peripheral sympathetic nerve activity and found that it increased in correlation with PEEP level. They considered that the mechanism might be a baroreflex activation of pulmonary mechanoreceptors with the vagal afferents. Although little is known regarding the effect of PEEP on HRV and SAPV, some studies on continuous positive airway pressure (CPAP) have been documented. Török et al[26] examined healthy volunteers in spontaneous breathing using 10 cmH_2O CPAP through a mouthpiece. They found that application of CPAP significantly increased LF and TP, whereas HF did not change significantly. Butler et al[5] examined congestive heart failure patients with spontaneous breathing using 10 cmH_2O CPAP. They found that TP, HF, and LF in HRV analysis increased during CPAP. Fraizer et al[6] investigated canines with normal ventricular function. They analyzed HRV in response to 3 different ventilatory conditions: pressure support at 10 cmH_2O, CPAP at 10 cmH_2O, and a combination of the two, each at 10 cmH_2O (pressure support plus CPAP). They found that LF increased and HF decreased during pressure support plus CPAP; however, there was no change in HRV parameters during pressure support only. They considered that the reduced preload generated by CPAP activated cardiac sympathetic activity and depressed parasympathetic activity. In the present study, we found that 5 cmH_2O PEEP had no effect on mean R-R interval, systolic arterial pressure, ICP, CPP, and SEF90, as seen at M1 and M2 in group P. There was also insignificant effect on these variables and plasma catecholamine in both groups. The changes in LF and HF from M3 to M6 reflected the effect of PEEP on CANS activity during brain damage. Both LF and HF in group P seemed to be lower than those in group Z from M3 to M6. As LF at M6 in group P was comparable to that at M5 in group Z, it was considered that CANS activity was depressed and could not excite until M6 in group P. At M6, SEF90 became absent in all animals, showing brain death. The intergroup differences in LF of both HRV and SAPV and the difference in HF of HRV seemed to show that the cardiac sympathetic and parasympathetic activity was depressed by 5 cmH_2O PEEP.

Hypercapnia itself aggravates brain function by increasing ICP, and increases cardiac sympathetic activity via a chemoreceptor[11]. $PaCO_2$ increased equally in the progression of brain damage in both groups in the present study. We thus assumed that $PaCO_2$ produced no difference between the groups.

General anesthesia was inevitable to induce brain damage in animal experiments. Both inhalation and intravenous anesthetics were applicable for this purpose. We utilized an intravenous anesthetic for induction of anesthesia and an inhalation anesthetic for maintenance of anesthesia, since end-tidal concentration of the agent could be easily monitored to maintain the same depth of anesthesia throughout the procedure. In the pre-

sent study, we used isoflurane and carefully monitored its end-tidal concentration. Isoflurane anesthesia can reduce in LF and HF in HRV analysis and baroreflex sensitivity[17]. However, we assumed that the differences observed between the groups were not induced by isoflurane because the subjects were maintained with same concentration of the anesthetic in both groups.

In the present study, a marked difference was observed in the results of microsphere counting between the groups. We could not rule out a mechanism in which continuous 5 cmH_2O PEEP itself depressed CANS activity. Thus, aggravation of brain function by PEEP might not be the only mechanism producing intergroup differences in HRV and SAPV parameters. We therefore concluded that CANS activity was depressed in the animals with acute brain damage during mechanical ventilation using 5 cmH_2O PEEP.

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