FULL PAPER ***Surgery***

The Effect of Electro-Acupuncture Stimulation on Rhythm of Autonomic Nervous System in Dogs

Yuuya KIMURA[1)] and Shigeo HARA[2)]*

[1)]Department of Small Animal Surgery, Faculty of Agriculture, 3-18-8 Ueda, Morioka, Iwate, 020-8550 and [2)]Morioka Veterinary Clinic Center, Branch of Center for Regional Collaboration in Research and Education, Iwate University, 2–2–7 Higashi Aniwa, Morioka, Iwate, 020–0824, Japan

(Received 9 November 2006/Accepted 10 December 2007)

ABSTRACT. Effects of electro-acupuncture (EA) stimulation on the rhythm of the autonomic nervous system in dogs were studied. Six healthy beagles were used in this study. Each dog was separately kept in a cage, and repeatedly exposed to light for 12 hr and dark for 12 hr alternately. Fixed subject dogs were stimulated by use of 5-V, 250-μsec, 2-Hz biphasic square pulses for 15 min at the *Xuan Shu* (GV-5) and *Bai Hui* (GV-20) points on the spine. After EA stimulation, electrocardiogram was recorded for 24 hr. From the electrocardiogram data, the heart rate (HR), coefficient of variation in the R-R intervals (CVRR; index of autonomic nervous activity), power of high frequency component (HF; index of vagal nervous activity), and ratio of powers of the low and high frequency components (LF/HF; index of sympathetic nervous activity) were obtained. Cosinor analysis demonstrated that these indices exhibited a significant rhythmicity ($P<0.05$), irrespective of EA stimulation. In LF/HF, EA stimulation advanced the acrophase (from 22:55 to 21:33, $P=0.012$), and elevated the midline-estimating statistic of rhythm (from 0.653 to 0.725, $P=0.006$). However, there was no significant difference in HR, CVRR, or HF. In conclusion, EA stimulation markedly influenced the rhythm of sympathetic nervous system in dogs.

KEY WORDS: autonomic nervous system, circadian rhythm, electro-acupuncture, heart rate variability.

J. Vet. Med. Sci. 70(4): 349–352, 2008

Electro-acupuncture (EA) is one of the techniques of acupuncture within traditional Chinese medical practice. Acupuncture produces a variety of therapeutic effects in analgesia, anti-inflammation and the management of insomnia in humans [20]. Recently, acupuncture has been used for treating acute pains, paralysis, lameness, gastrointestinal disorder and uropathy in veterinary practice [5]. However, the mechanisms are still unclear.

Autonomic nervous system constantly controls and monitors many functions in the body. Sympathetic and parasympathetic nervous regulation of the heart contributes to the characteristic frequency in the heart rate variability (HRV) [1]. Because data acquisition via an electrocardiogram (ECG) is easy, frequency-domain analysis of HRV enables a sophisticated and noninvasive methodology for monitoring the autonomic regulation of the heart. It has been well established that HRV can be separated into high-frequency and low-frequency components by oscillation frequency [17]: the vagal activity is a major contributor to the power of high-frequency component (HF), while the power of low-frequency component (LF) reflects both sympathetic and vagal activities. Some investigators have suggested that the ratio of LF and HF (LF/HF) serves as an index of the sympathetic activity. In addition, the coefficient of variation in the R-R intervals (CVRR), defined as the ratio of the standard deviation of the R-R intervals to their average value, has been used for quantitatively evaluating the activity of the overall autonomic nervous system [11, 16].

Although the effects of acupuncture on the autonomic nervous system have been researched [10, 18], continuous effects lasting for a long period are still to be investigated. When individual rhythmicities are different, the simple average of time-series values may give a wrong direction. Therefore, a cosinor method, developed as a basic method for quantitatively analyzing rhythmicity, is used for evaluating the long-period effects [8, 9, 14].

The aim of this study was to quantitatively evaluate the effects of EA stimulation on the rhythmicity of the autonomic nervous system in dogs. When the long-period effects of EA are clarified, acupuncture is expected to be used for treating a wide range of disorders, such as insomnia and narcolepsy.

MATERIALS AND METHODS

The study was approved by the Animal Research Committee of Iwate University. Six healthy beagles (male, 8–13 kg, 2 years old) were used in this study. Each dog was separately kept in a cage (47 × 75 × 45 cm) in a climate-controlled room (approximately 22°C). All work and care were performed once a day between 6:30 and 7:00. Other than those above, nobody entered the room. Lights were automatically turned on at 23:45 and turned off at 11:45. The subject dogs were exposed to light for 12 hr and kept in dark for 12 hr, repeatedly.

The subject dogs were dressed in a jacket that made for a Holter recorder (QR2100; 88 × 62 × 23 mm, 138 g, FUKUDA ME. Co., Ltd., Japan) for 24-hr ambulatory ECG monitoring with a canine Elizabethan collar around the

* CORRESPONDENCE TO: HARA, S., Morioka Veterinary Clinic Center, Center for Regional Collaboration in Research and Education, Iwate University, Japan.
e-mail: shara@iwate-u.ac.jp

neck. They were subjected to an acclimation period for five days. The subject dogs were left in a washout period for at least three days after each treatment.

The dog was tied to a stainless-steel fixing tool (42 × 25 × 34 cm) with cords for 15 min (Restraint group). In the restraint state, EA stimulation was provided at the acupoints of *Xuan Shu* (GV-5) and *Bai Hui* (GV-20) for 15 min (Restraint + EA group). *Xuan Shu* is located in the gap between the final thoracic and first lumbar vertebra (T13-L1), while *Bai Hui* is located in the gap between the final lumbar and first sacral vertebra (L7-S1). The acupuncture needles were inserted into the acupoints to a depth of about 2 cm, and electric stimulation was provided through the needles by an EA treatment device (ES-160; Itoh Ultrashort-wave Co., Ltd., Japan). The output rectangular wave was kept at 5 V and 250 μsec with frequency of 2 Hz. The ECG recording was begun after completing each treatment.

Data obtained in the 24-hr ambulatory ECG monitoring were converted by an ECG analysis software (HS1000 system; FUKUDA ME. Co., Ltd., Japan). For accuracy, the lower limit of the analytic rates of ECG was set at 95%. When the analytic rates were less than 95%, the data were rejected.

The heart rate (HR) and CVRR were obtained from raw ECG data. Spectral analysis was performed using the R-R intervals for the respective points. A 5-min mean of the power spectrum of longitudinal HRV was obtained by fast Fourier transformation. From the spectrum, LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz) were picked up, and then LF/HF was calculated. The 5-min mean data (HR, CVRR, HF and LF/HF) were fitted to 24-hr cosine curves by a least squares method via a longitudinal data analysis system (MemCalc/CHIRAM; GMS Co., Ltd., Japan).

The 24-hr best-fitted cosine curves were represented as a midline-estimating statistic of rhythm (MESOR), acrophase and amplitude. These parameters were evaluated by zero amplitude tests; the rhythmicity is statistically significant (P<0.05) when the 95% confidence ellipse does not overlap the origin of a polar coordinate system. When the rhythmicity was significant, the parameters of rhythm were compared using a paired t-test. Difference between Restraint and Restraint + EA groups was statistically analyzed (P<0.05).

RESULTS

Data obtained from five out of six subject dogs were used for analysis. ECG data obtained from the rest one was dropped due to a small analytic rate. The final analytic rates were 99.4 ± 0.37% in the Restraint group, and 98.1 ± 1.57% in the Restraint + EA group (mean ± standard error).

HR became high after treatment, and then decreased gradually. Contrary, CVRR, HF and LF/HF decreased after treatment, and then increased to a maximum in dark condition. The variation in the parameters was decreased in the Restraint + EA group.

The cosinor parameters are represented as mean ± standard error (Table 1). In order to examine the rhythmicity of the indices, the respective amplitude and acrophase were plotted in a polar coordinate system (Fig. 1). Because the standard errors were decreased by EA stimulation, the 95%

Table 1. Cosinor parameters of 24-hr transition of HR and HRV

	Restraint	Restraint + EA
HR		
MESOR	73 ± 3	71 ± 3
Amplitude	10 ± 3	9 ± 2
Acrophase	9:13 ± 88 min	10:15 ± 39 min
CVRR		
MESOR	31.2 ± 2.2	32.9 ± 1.8
Amplitude	5.3 ± 1.0	4.7 ± 1.2
Acrophase	21:37 ± 65 min	21:41 ± 29 min
HF		
MESOR	482 ± 49	500 ± 45
Amplitude	98 ± 19	88 ± 20
Acrophase	22:30 ± 121 min	21:13 ± 52 min
LF/HF		
MESOR*	0.653 ± 0.078	0.725 ± 0.089
Amplitude	0.258 ± 0.033	0.211 ± 0.021
Acrophase*	22:55 ± 52 min	21:33 ± 49 min

*: P<0.05.

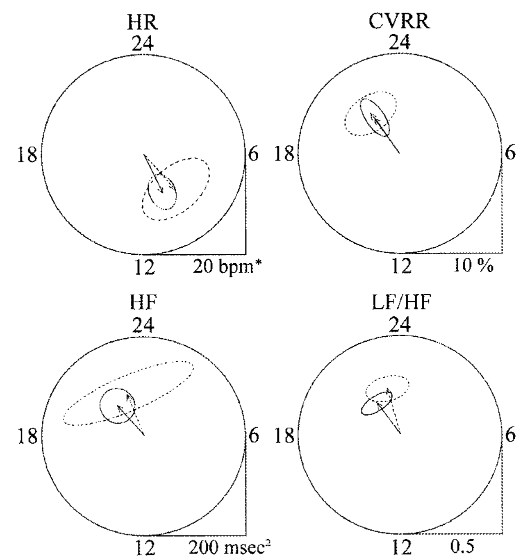

Fig. 1. Trend of autonomic nervous system of heart analyzed by cosinor method. The values presented in a polar coordinate system. The dashed data is result of the Restraint group, while the solid one is result of the Restraint + EA group. Period length (24 hr) is shown as a full circle. Length of vector indicates the amplitude, and angle of the vector from phase onset (00:00) indicates the acrophase. The ellipse centered on the end of the vector indicates the 95% confidence region for amplitude and acrophase. The 95% confidence ellipse does not overlap with the center of the polar coordinate system, which indicate significant circadian rhythmicity (P<0.05). *: Beat per minut.

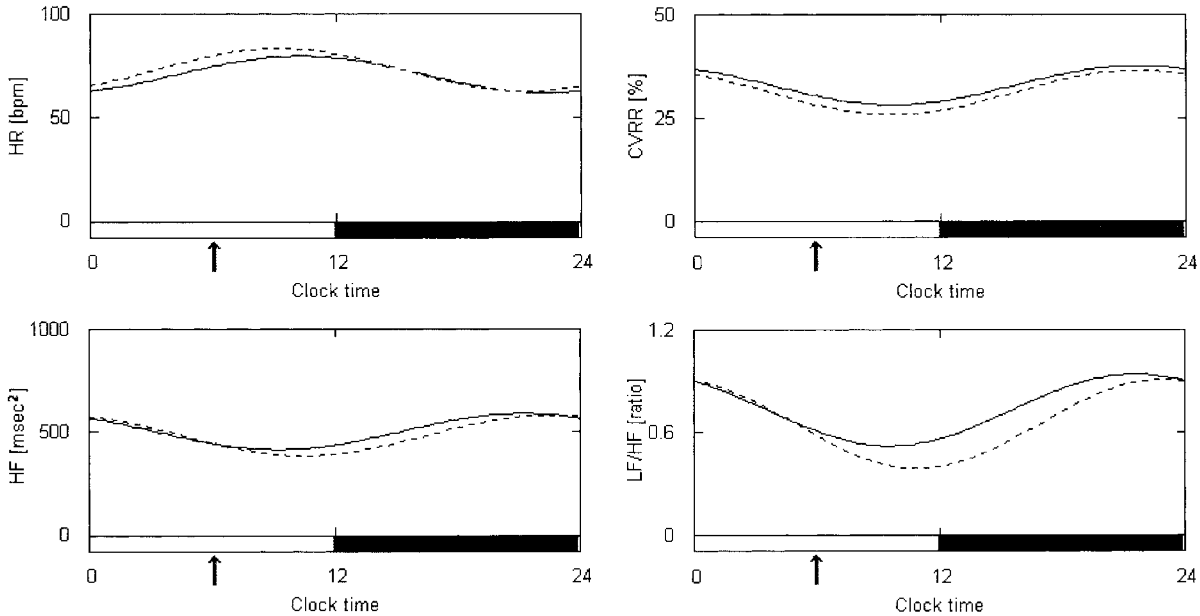

Fig. 2. Twenty-four hour cosine curve fitting to actual obtained values. Dashed line is indicate of the Restraint group, and solid line is indicate of the Restraint + EA group. The white and black bars bottom the graph indicate the duration of the light and dark phases of the light / dark cycle. Dogs were treated around the arrow. The curve of the LF/HF dramatically rises and advances.

confidence ellipses were diminished for the Restraint + EA group. Every 95% confidence ellipse did not overlap with the center of the polar coordinate system. Therefore, the indices indicated statistical significance in rhythmicity (P<0.05).

Best fitting 24-hr cosine curves were plotted in a rectangular coordinate system (Fig. 2). Comparing several parameters of rhythm by paired t-test, the transition of LF/HF gave significant advance in acrophase (from 22:55 ± 52 to 21:33 ± 49 min, P=0.012) and increase in MESOR (from 0.653 ± 0.078 to 0.725 ± 0.089, P=0.006). No significant difference in the other indices was found.

DISCUSSION

Significant circadian rhythms in the HR and HRV were observed in healthy beagles. HR was increased in light condition and decreased in dark condition, while the indices of HRV were decreased in light condition and increased in dark condition. EA stimulation conduced to a significant change in LF/HF rhythm.

The changes in autonomic nervous system can be quantitatively assessed by frequency domain analysis of HRV. Effects of autonomic pharmacologic blockades have indicated that the HF variation is a marker of efferent vagal input to the heart, and LF/HF is an indicator of sympathetic tone [1, 11, 16, 17]. Autonomic nervous activity exhibits diurnal fluctuation. Results obtained in studies of 24-hr transition of HRV indicated that relative sympathetic dominance was observed in the daytime, while parasympathetic dominance in the nighttime [12, 15]. Furthermore, sleep is likely to involve with transition of autonomic nervous activity. Furlan *et al.* reported increase in HF and decrease in LF/HF during sleep [7]. Also, Burgess *et al.* have demonstrated that the parasympathetic nervous activity was mostly influenced by the circadian system, and the sympathetic nervous activity was influenced by the sleep system in particular [4].

Indeed, acupuncture is employed for treating insomnia in humans [2]. Because insomnia is one of the circadian disorders, adjustment of the biorhythm is important in treating insomnia. The hypothalamus has been reported to be involved in the circadian pacemaking in mammals [3], and its malfunction could cause sleep disorders [13]. Recent studies with the magnetic resonance imaging (MRI) demonstrated change in the hypothalamus in consequence of acupuncture [6, 19]. Therefore, acupunctural effect could be related to improvement of insomnia by adjusting functions of the hypothalamus. Reduced 95% confidence ellipse observed in the EA stimulated dogs suggested that EA could regulate the biorhythm in dogs.

In conclusion, our results suggest that EA stimulation at the *Xuan Shu* (GV-5) and *Bai Hui* (GV-20) points markedly advance and activate rhythm of sympathetic nervous activities in dogs. EA has been widely employed in traditional Chinese medical practice. It could be an efficacious therapeutic technique for treating the circadian disorders, such as insomnia and narcolepsy.

ACKNOWLEDGMENT. We thank FUKUDA ME. Co., Ltd. and GMS Co., Ltd. for allowing us to use the instruments.

REFERENCES

1. Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C. and Cohen, R. J. 1981. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* **213**: 220–222.
2. Attele, A. S., Xie, J. T. and Yuan, C. S. 2000. Treatment of insomnia: an alternative approach. *Altern. Med. Rev.* **5**: 249–259.
3. Buijs, R. M., van Eden, C. G., Goncharuk, V. D. and Kalsbeek, A. 2003. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J. Endocrinol.* **177**: 17–26.
4. Burgess, H. J., Trinder, J., Kim, Y. and Luke, D. 1997. Sleep and circadian influences on cardiac autonomic nervous system activity. *Am. J. Physiol.* **273**: H1761–1768.
5. Chan, W. W., Chen, K. Y., Liu, H., Wu, L. S. and Lin, J. H. 2001. Acupuncture for general veterinary practice. *J. Vet. Med. Sci.* **63**: 1057–1062.
6. Chiu, J. H., Chung, M. S., Cheng, H. C., Yeh, T. C., Hsieh, J. C., Chang, C. Y., Kuo, W. Y., Cheng, H. and Ho, L. T. 2003. Different central manifestations in response to electroacupuncture at analgesic and nonanalgesic acupoints in rats: a manganese enhanced functional magnetic resonance imaging study. *Can. J. Vet. Res.* **67**: 94–101.
7. Furlan, R., Guzzetti, S., Crivellaro, W., Dassi, S., Tinelli, M., Baselli, G., Cerutti, S., Lombardi, F., Pagani, M. and Malliani, A. 1990. Continuous 24-hr assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation* **81**: 537–547.
8. Giuseppe, P. and Giovanni, C. 2002. Biological rhythm in livestock. *J. Vet. Sci.* **3**: 147–157.
9. Halberg, F., Cornelissen, G., Katinas, G., Syutkina, E. V., Sothern, R. B., Zaslavskaya, R., Halberg, F., Watanabe, Y., Schwartzkopff, O., Otsuka, K., Tarquini, R., Frederico, P. and Siggelova, J. 2003. Transdisciplinary unifying implications of circadian findings in the 1950s. *J. Circadian Rhythms* **1**: 2.
10. Li, Z., Wang, C., Mak, A. F. and Chow, D. H. 2005. Effects of acupuncture on heart rate variability in normal subjects under fatigue and non-fatigue state. *Eur. J. Appl. Physiol.* **94**: 633–640.
11. Malliani, A., Pagani, M., Lombardi, F. and Cerutti, S. 1991. Cardiovascular neural regulation explored in the frequency domain. *Circulation* **84**: 482–492.
12. Matsunaga, T., Harada, T., Mitsui, T., Inokuma, M., Hashimoto, M., Miyauchi, M., Murano, H. and Shibutani, Y. 2001. Spectral analysis of circadian rhythms in heart rate variability of dogs. *Am. J. Vet. Res.* **62**: 37–42.
13. Moore, R. Y. 1997. Circadian rhythms: basic neurobiology and clinical applications. *Annu. Rev. Med.* **48**: 253–266.
14. Nagayama, H. 1991. Methods for Analysis of Circadian Rhythms. *Brain Sci. Ment. Dis.* **2**: 789–793 (In Japanese).
15. Nakagawa, M., Iwao, T., Ishida, S., Yonemochi, H., Fujino, T., Saikawa, T. and Ito, M. 1998. Circadian rhythm of the signal averaged electrocardiogram and its relation to heart rate variability in healthy subjects. *Heart* **79**: 493–496.
16. Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., Sandrone, G., Malfatto, G., Dell'Orto, S., Piccaluga, E. *et al.* 1986. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ. Res.* **59**: 178–193.
17. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* **93**: 1043–1065.
18. Wang, J. D., Kuo, T. B. and Yang, C. C. 2002. An alternative method to enhance vagal activities and suppress sympathetic activities in humans. *Auton. Neurosci.* **100**: 90–95.
19. Wu, M. T., Hsieh, J. C., Xiong, J., Yang, C. F., Pan, H. B., Chen, Y. C., Tsai, G., Rosen, B. R. and Kwong, K. K. 1999. Central nervous pathway for acupuncture stimulation: localization of processing with functional MR imaging of the brain-preliminary experience. *Radiology* **212**: 133–141.
20. Yi, P. L., Tsai, C. H., Lin, J. G., Liu, H. J. and Chang, F. C. 2004. Effects of electroacupuncture at 'Anmian (Extra)' acupoints on sleep activities in rats: the implication of the caudal nucleus tractus solitarius. *J. Biomed. Sci.* **11**: 579–590.