NOTE *Surgery*

Effects of Atipamezole and Naloxone on Electroencephalographic Spectral Edge Frequency 95 in Dogs Sedated by Acupuncture at GV20 and Yintang Point

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ABSTRACT. The purpose of this study was to evaluate the antagonism of acupuncture-induced sedation by electroencephalographic spectral edge frequency (SEF) 95 in 10 healthy intact male Miniature Schnauzer dogs (4.2–6.1kg; 2–3 years old) without neurological disorder. The GV20 and Yintang acupoints were administered for 20 min. While the dogs were conscious, SEF 95 baseline values were recorded at 2-min intervals for 4 min. Then acupuncture was administered at the GV20 and Yintang acupoints. During the acupuncture procedure, the SEF 95 values were recorded at 2-min intervals for 10 min. Subsequently, antagonist drugs, naloxone and atipamezole, were administered through the cephalic vein. The SEF 95 values were then measured again at 2-min intervals for 10 min. Those values were found to be significantly increased after administration of atipamezole in dogs sedated by acupuncture at the GV20 and Yintang acupoints ($p<0.05$). However, the SEF 95 values in the naloxone groups did not show any significant changes before and after administration of the antagonist. It was concluded that sedation induced by acupuncture at the GV20 and Yintang acupoints might be partially associated with the α_2-adrenergic system.

KEY WORDS: acupuncture, antagonist, spectral edge frequency 95.

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Acupuncture, among available therapies, is potentially an important adjuvant for pain and mental control. Indeed, it is increasingly practiced in common medical setting: physicians in both human and animal medicine have been increasing the use of acupuncture [3]. Among the acupuncture points for sedation and analgesia, it has been reported that the acupuncture points GV20 and Yintang can induce sedation and provide relief from stress in humans [14, 16]. We also reported in a previous study, that acupuncture at the GV20 and Yintang acupoints induced sedation in dogs [13]. Although heart rate, blood pressure, respiratory rate, muscle relaxation, and ocular signs have been used to assess sedative conditions, electroencephalography (EEG) may be a simple valuable tool in the evaluation of central nervous system (CNS) activity [8]. Spectral edge frequency (SEF) 95 is one of the important evaluation parameters of EEG. The exact mechanism of the action and sedative effect of acupuncture has yet to be established. It has been reported that the anxiolytic and sedative effects of acupuncture might be related to secretion of endogenous neurotransmitters [7]. Especially, it has been suggested that the opioid system and the adrenergic system might play the key roles in the mediation of the effects of acupuncture in clinical and experimental data [19]. In examining this issue, the present study hypothesized that the sedative effect of acupuncture at the GV20 and Yintang acupoints could be effectively reversed by administration of antagonist(s): naloxone (opioids antagonist) or atipamezole (α_2-adrenergic receptor antagonist) intravenously. The bio-electrical changes in the brain resulting from those administrations were analyzed by SEF 95 as in the previous study [13]. The purpose of the present study was to determine whether the sedative effect of acupuncture is related to the activation of opioid receptors and α_2-adrenoceptors or not using electroencephalographic SEF 95.

Ten healthy intact male Miniature Schnauzer dogs (4.2–6.1 kg, 1–2 years old) without neurological disorder were used in the study. Animals of the same breed were chosen in order to standardize the anatomic sites of electrode-placement. The dogs were repeatedly used with a washout period of 2-week. The acupoints GV20 and Yintang were used. The acupoint GV20 is located at the vertex on the midline, the Yintang point is midway between the medial ends of the two eyebrows. Hair over the acupoints was clipped before the experiment. The dogs were assigned randomly to 5 study groups (n=5/group, respectively). Five study groups were a control group and four acupuncture groups (the GV20 + naloxone group, the Yintang + naloxone group, the GV20 + atipamezole group and the Yintang + atipamezole group). The control group, which did not receive acupuncture but did receive the antagonists, was evaluated the SEF 95 value resulting from the drug alone. This study adhered to the strict guidelines established in the "Guide for the Care and Use of Laboratory Animals" of Seoul National University (Seoul, South Korea). Computerized EEG equipment (Model: QEEG-8, LXE3208, LAXTHA Inc., Korea) was used and the EEG electrodes were positioned according to a procedure similar to that followed in the previous study [13]. The low-frequency filter was set at 2 Hz, and the high-frequency setting was 50 Hz. For the administration of the antagonists prior to experiment, an intravenous catheter (D&B-Cath, Sin Dong Bang Medical Co., Seoul, Korea) with extension line were placed into the cephalic vein on the

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left side. The duration of the experiment was 25 min. While the dogs were conscious SEF 95 baseline values were recorded at 2-min intervals for 4 min. Then, acupuncture was manually administered at the GV20 and Yintang acupoints using sterilized needles (32 gauge, 15 mm length, Haeng Lim Seo Won, Seoul, Korea). The needles were inserted about 0.5 to 1 cm into the skin, and left for 20 min without manipulation. During the acupuncture procedure, SEF 95 values were recorded at 2-min intervals for 10 min. Subsequently, the antagonist drugs, 0.04 mg/kg of naloxone (Naloxone HCl, Keukdong pharm Co., Ltd, Seoul, Korea) and 0.2 mg/kg of atipamezole (Antisedan, Orion Pharma, Finland), were administered through the cephalic vein. The dosage of naloxone was in the line with a previous study and that of atipamezole was consistent with the dosage of medetomidine commonly administered in dogs [18]. The SEF 95 values were then measured again at 2-min intervals for 10 min (c-g). The recordings were taken at a time points a- to g shown in Fig. 1.

Data values were expressed as the mean ± standard deviation (SD) of the mean. Analysis of variance (ANOVA) was used to compare the SEF 95 values of the normal baseline versus those of antagonist administration, the baseline versus acupuncture and acupuncture versus antagonist administration, respectively. For each test, $p<0.05$ was considered significant.

In the control group, the SEF 95 values recorded prior to and after agonist administration were similar for both drugs as time passed. The SEF 95 values did not significantly change with the administration of the antagonists (Fig. 2). While the dogs were conscious, the SEF 95 values recorded in the GV20 + naloxone group, the Yintang + naloxone group, the GV20 + atipamezole group, and the Yintang + atipamezole group were similar. The means of the values (± SD) were 44.99 ± 0.62 Hz (GV20 + naloxone group), 44.49 ± 0.48 Hz (Yintang + naloxone group), 44.57 ± 0.75 Hz (GV20 + atipamezole group), and 44.14 ± 0.31 Hz (Yintang + atipamezole group), respectively (Figs. 3, 4). However, the values were significantly reduced after acupuncture, by which time the dogs became calm and showed reduced movement, in all of the groups ($p<0.05$). The results were similar those of the previous studies [13, 14, 16]. During acupuncture, the means of the values were 41.56 ± 2.29 Hz (GV20 + naloxone group), 41.57 ± 1.20 Hz (Yintang + naloxone group), 39.01 ± 2.61 Hz (GV20 + atipamezole group), and 38.90 ± 1.69 Hz (Yintang + atipamezole group), respectively. The mean values of the atipamezole groups were lower than those of the naloxone groups. And after the antagonist treatment, the values showed a significant increase only in the atipamezole treatment groups. The dogs of the atipamezole treatment groups showed severe agitation as well as restlessness. Six minutes after the atipamezole treatment, the means of the values were 44.20 ± 0.90 Hz (GV20 + atipamezole group), and 44.59 ± 1.11 Hz (Yintang + atipamezole group), respectively (Fig. 4). However, the values of the naloxone groups did not show any significant change (Fig. 3). The dogs of the naloxone treatment

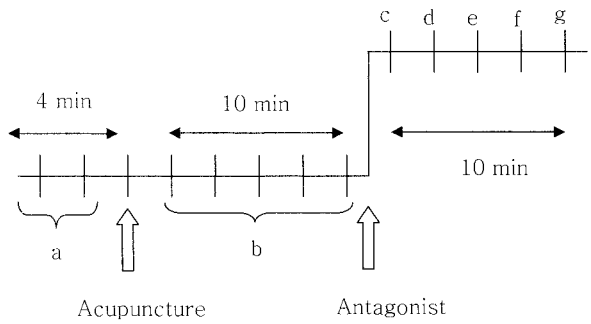

Fig. 1. Experimental procedure with different measuring-time points a: average value of 2 measured data during 4 min in conscious condition (before acupuncture); b: average value of 5 measured data during 10 min of acupuncture; c: the value 2 min after antagonist treatment; d: the value 4 min after antagonist treatment; e: the value 6 min after antagonist treatment; f: the value 8 min after antagonist treatment; g: the value 10 min after antagonist treatment.

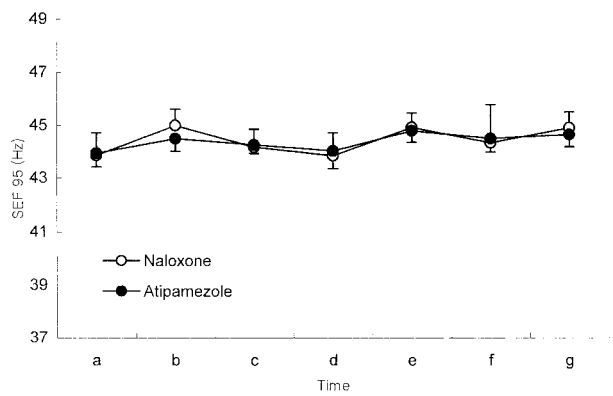

Fig. 2. SEF 95 values (mean ± SD) for conscious condition (a) and antagonist condition (b-g) recorded in the control group without acupuncture.

groups did not show any wakefulness. This result showed that the sedation induced by acupuncture at the GV20 and Yintang acupoints was effectively blocked by the α_2-adrenergic receptor antagonist, atipamezole.

According to the overall results, the SEF 95 values showed a significant increase after atipamezole administration. This demonstrates that the sedative effect of acupuncture might be at least related to a α_2-adrenoceptor associated neural component. This conclusion is in accordance with the findings of Chen [4] that yohimbine, a α_2-adrenergic receptor antagonist, can inhibit the effect of electroacupuncture. The α_2-adrenoceptor in CNS modulates perception of pain and levels of sedation [17]. In veterinary medicine, α_2-adrenoceptor agonists have been used for sedation, analgesia, anxiolysis, and sympatholysis [1, 15]. Among the acupoints for sedation and analgesia, the acupuncture points GV20 and Yintang have been reported to be capable of inducing sedation and providing relief from stress in both dogs and humans [3, 13, 14]. Despite the manifest therapeu-

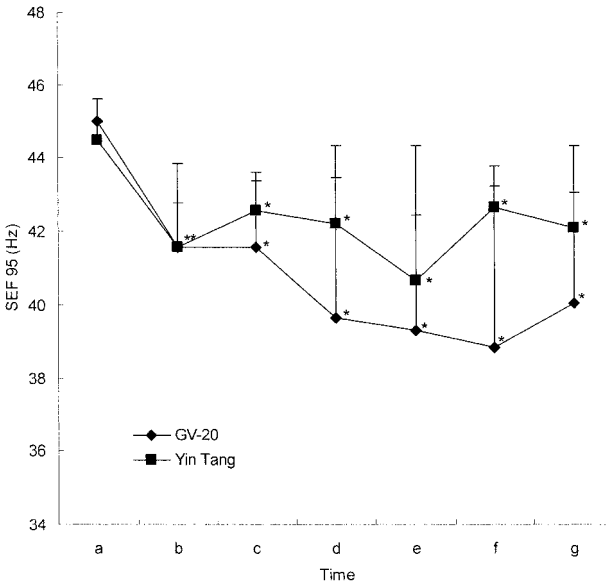

Fig. 3. SEF 95 values (mean ± SD) for conscious condition (a), acupuncture (b) and antagonist (c-g) recorded in two experimental groups (GV20 + naloxone and Yintang + naloxone). * Significantly different compared to a (conscious condition) as $p<0.05$.

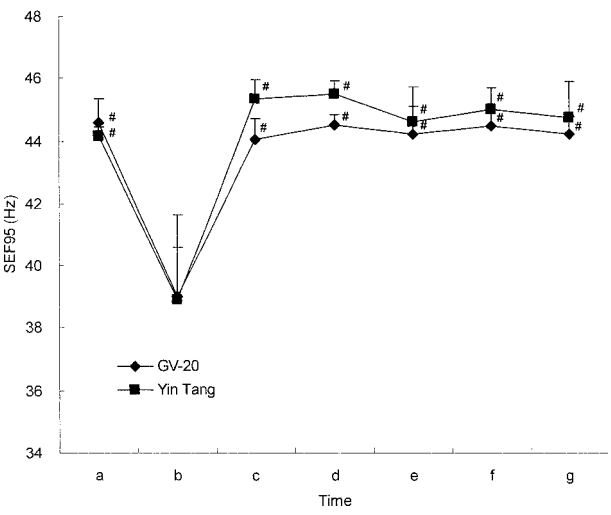

Fig. 4. SEF 95 values (mean ± SD) for conscious condition (a), acupuncture (b) and antagonist (c-g) recorded in 2 experimental groups (GV20 + atipamezole and Yintang + atipamezole). # Significantly different compared to b (acupuncture) as $p<0.05$.

tic benefit of acupuncture, the exact mechanism of the action and effectiveness of acupuncture has not been established [5, 11]. As the mechanism of acupuncture has been divided into several classifications, the opioid system or the adrenergic system have been considered to play important roles in mediating the effect of acupuncture [5, 6, 12, 20]. However, some scientists have suggested that the opioid antagonist naloxone has no effect on hypnotic analgesia [8]. In the present study even though the single parameter, SEF 95, was used to examine the sedative condition, the sedative effect of acupuncture at the GV20 and Yintang acupoints was not reversed by the opioid receptor antagonist but was reversed by the α_2-adrenoceptor antagonist. In relation to this issue, some neuro-pharmacologists have assessed the role of central norepinephrine (NE) in acupuncture [11]. It was reported that acupuncture reduced NE levels in certain brain areas as well as in the blood circulation [2]. Moreover the Influence of acupuncture has been observed in the lesion of the locus coeruleus (LC) [14]. The LC has been believed to be the major site of action for the sedative effects of the α_2-adrenoceptor agonist [15]. The α_2-adrenoceptor antagonists, such as atipamezole or yohimbine, increase the activity of LC neurons and heighten behavioral arousal. Based on these findings, the sedation induced by acupuncture in the present study might be associated with inhibition of LC in the brain. In conclusion, it is suggested that acupuncture-induced sedation is associated more with the α_2-adrenergic system than with the endogenous opioid system.

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