

## Skin Conductance Reflects Drug-Induced Changes in Blood Levels of Cortisol, Adrenaline and Noradrenaline in Dogs

Maki ISHIBASHI<sup>1)\*</sup>, Hideo AKIYOSHI<sup>1)</sup>, Toshie ISERI<sup>1)</sup> and Fumihito OHASHI<sup>1)</sup>

<sup>1)</sup>Department of Veterinary Clinical Medicine, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-ohrai-kita, Izumisano, Osaka 598-8531, Japan

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**ABSTRACT.** To verify availability of skin conductance (SC) as an indicator for the sympathetic nervous system (SNS) activity in dogs, the changes in SC and blood levels of stress-related hormones induced by drugs were compared. SC and cortisol, adrenaline and noradrenaline levels were measured in 5 dogs on 4 occasions with or without drug-induced sedation at 7-day intervals (no treatment, intramuscular medetomidine 0.01 mg/kg, intramuscular acepromazine 0.1 mg/kg and intravenous fentanyl 0.02 mg/kg). The fentanyl treatment produced significantly higher levels of SC and plasma cortisol and adrenaline compared with the other 3 treatments. The plasma noradrenaline level also tended to be higher following the fentanyl treatment. These results indicate that SC may reflect changes in the SNS activities in dogs.

**KEY WORDS:** nervous system, skin conductance, stress.

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Stress is the physical and physiological influence exerted on bodily functions by threatening incidents. Transient stress is normally controlled by physiological activities that maintain the body in a steady state. However, exposure to continual stress is harder to control and may cause stress-related diseases, such as atrophy of lymph nodes [1], peptic ulcers [16] and high blood pressure [22]. To prevent these diseases and improve the quality of life, it is important to elucidate the stress levels experienced by the body.

Traditional indicators of stress are stress-related hormones, such as cortisol and catecholamines [3, 5]. At first, stress is exhibited in the limbic system and is then transmitted to the rest of the body via 2 pathways: the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic-adrenal-medullary axis (SAM axis). The HPA axis activates the secretion of cortisol, whereas the SAM axis activates the secretion of catecholamines. Thus, the release of stress-related hormones can be used to evaluate the current stress level [3]. These hormones are contained in both blood and saliva [12]; thus, it is common to measure cortisol and catecholamine levels in blood and saliva samples. This measurement method, however, can be problematic, as the manner in which the sampling is performed can induce more stress, especially in animals, and affect the results. Moreover, the sample analysis is complicated and time-consuming. Therefore, researchers have been looking for a noninvasive and easy-to-use indicator of stress in animals.

Recently, skin conductance (SC) has been used as an

indicator of stress in humans [6, 7, 14, 18]. Cells in the stratum corneum each have an electrical double layer, which causes polarization of the skin to elicit a capacitance under the influence of an electric field. The electrical impedance of the stratum corneum is short-circuited by resistive channels that are located between the cells [20]. The activity of sweat glands, which are under the control of the sympathetic nervous system (SNS), changes the ion permeability of the resistive channels and influences electrical impedance. When stress increases, SNS stimulation activates the sweat glands, which in turn decreases electrical impedance and leads to an increase in SC [6, 17]. SC is measured by passing a small amount of electrical current through electrodes that are applied to skin [8]. This indicator is noninvasive and easier than traditional ones. In dogs, however, it is not clear whether SC reflects SNS activity like humans and can be used as an indicator of stress.

Some drugs induce changes in activities of the central nervous system (CNS) and autonomic nerve system (the SNS and/or parasympathetic nerve system). Medetomidine and acepromazine produce CNS depression and suppression of the SNS activity by decreasing release of catecholamines in dogs [21]. In contrast, fentanyl has neurohormonal effects similar to stress, such as increases in plasma concentrations of catecholamines and cortisol when administered to pain-free dogs [2].

The purpose of this study was to evaluate whether SC reflects the degree of SNS activity in dogs. We compared SC and blood levels of cortisol, adrenaline and noradrenaline in dogs with or without changes in the SNS activity induced by medetomidine, acepromazine or fentanyl.

Five healthy sexually intact female beagles aged 1 year and weighing 9.5–12 kg were included in the study. The dogs were accustomed to being restrained, having blood samples drawn and taking medications. They were housed separately in individual cages, in which the temperature was

\*CORRESPONDENCE TO: ISHIBASHI, M., Department of Veterinary Clinical Medicine, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-ohrai-kita, Izumisano, Osaka 598-8531, Japan.

e-mail: sq205004@edu.osakafu-u.ac.jp

maintained at  $23 \pm 1^\circ\text{C}$  and the light:dark rhythm was controlled in a 12:12 hr cycle. Feeding was twice a day, at 10:00 and 16:00, and water was available freely. Study protocols were approved by the Animal Care and Use Committee of Osaka Prefecture University.

SC and plasma cortisol, adrenaline and noradrenaline levels were measured in each of the dogs on 4 occasions with or without drug-induced sedation at 7-day intervals. On each occasion, the dogs received each of the following treatments: 1) no treatment (control), 2) an intramuscular injection (IM) of medetomidine (0.01 mg/kg), 3) an IM of acepromazine (0.1 mg/kg) and 4) an intravenous injection (IV) of fentanyl (0.02 mg/kg). SC was measured when the dogs were lying down and demonstrated a sedative level with a Ramsey sedation score of 2 to 3, which indicated that the dogs were oriented, calm and cooperative [11], except for the control group. SC was measured in awake dogs in the control group. Measurements were performed by using an instrument for measuring SC in dogs (PS-IMP001<sup>®</sup>, Live Aid Co., Ltd., Kanazawa, Japan) in a quiet space isolated from extraneous activity. Figure 1 shows the mechanism of this instrument. Electrodes (Echorode III<sup>®</sup>, ECG electrode, Fukuda Denshi Co., Ltd., Tokyo, Japan) were applied to the metacarpal pads, which contain sweat glands, and a pulse of 3 V was applied. This pulse created an electric wave that was amplified by the measurement apparatus and transformed into a digital value. This value was then substituted for the following equation to calculate SC [20]:  $y = (7,056x)^{-0.996} \times 100$ . The x value was the digital value obtained from measurements (k $\Omega$ ), and the y value was the SC (%). This equation was obtained based on the values measured by connecting many forms of resistance to the instrument. Measurements were performed 5 times for each treatment. Figure 2 shows the state of SC measurements in dogs.

Immediately after the SC measurements, approximately 7 ml of venous blood was collected from the cephalic vein. Of this, approximately 2 ml was placed into a tube with serum separating medium and centrifuged at 3,000 rpm for 10 min at  $4^\circ\text{C}$ . The serum layer was removed and stored at  $-80^\circ\text{C}$ . This serum was used to analyze cortisol level via an electrochemiluminescence immunoassay. The remaining 5 ml of sampled blood was injected into a tube containing EDTA-2Na and centrifuged at 3,000 rpm for 10 min at  $4^\circ\text{C}$ . The plasma layer was removed and stored at  $-80^\circ\text{C}$  until it was used to measure adrenaline and noradrenaline levels via high-performance liquid chromatography. These analyses of stress-related hormone levels were performed by a private clinical laboratory (Japan Clinical Laboratories, Inc., Osaka, Japan).

Effects of all treatments were cross-compared in each dog by using ANOVA and Dunnett's tests. Values of  $P < 0.05$  were considered significant.

The sedation level was evaluated as a score of 1 in the dogs with no treatment and as scores of 2 to 3 in the dogs administered drugs. From the ANOVA test, there were significant differences among the 4 treatments in stress-related hormone levels and SC ( $P < 0.01$ ). The results of the Dunnett's test showed that cortisol and adrenaline levels and SC in the

fentanyl treatment group were significantly higher than in the control ( $P < 0.01$ ) (Fig. 3a, 3b and 3d). The noradrenaline level was significantly lower in the medetomidine treatment group ( $P < 0.05$ ) and significantly higher in the fentanyl treatment group than in the control ( $P < 0.01$ ) (Fig. 3c).

These results from the present study were consistent with the abovementioned catecholamine responses to each drug. Catecholamines are secreted from the SAM axis, which includes the SNS; therefore, SC is considered to be directly reflective of SNS activity in dogs. In humans, Storm *et al.* [18] reported that SC changed with catecholamine fluctuations when subjects underwent whole body anesthesia for an operation, which is consistent with our results. Two different types of sympathetic nerve fibers are known to exist: fibers with noradrenaline in the postganglionic synapses and those with acetylcholine in the postganglionic synapses. Fibers that release acetylcholine innervate the sweat glands; thus, the activity of sweat glands is considered indicative of cholinergic activity [8, 18]. However, in a study of the functional activity of sweat glands in dogs, sweat increased when dogs were administered either acetylcholine or catecholamines [19]. This result suggested that sweat glands in dogs were innervated by the SNS, but may also reflect catecholamine activity.

In this study, we measured SC when the dogs demonstrated a sedative level with a Ramsey sedation score of 2 to 3 with lying down in the drug treatment groups in order to produce certain changes in the SNS activities. Administrations of 0.01 mg/kg medetomidine IM and 0.01 mg/kg acepromazine IM have been commonly used for dogs to achieve sedation with lying down position in veterinary practice. Kamata *et al.* [10] reported that 4 of 6 dogs were lying down following an administration of 0.02 mg/kg fentanyl IV without severe bradycardia and respiratory depression and that 6 of 6 dogs were lying down following 0.04 mg/kg fentanyl IV with severe bradycardia and respiratory depression. Väisänen *et al.* [21] reported that medetomidine and butorphanol (0.02 and 0.2 mg/kg, respectively, IM) and acepromazine and butorphanol (0.05 and 0.2 mg/kg, respectively, IM) produced significant changes in plasma concentrations of cortisol, adrenaline, and noradrenaline in dogs. Ambrisko *et al.* [2] reported that 0.01 mg/kg fentanyl IM increased the plasma concentrations of cortisol, adrenaline and noradrenaline in dogs. In this study, we adopted the dosages of medetomidine, acepromazine and fentanyl based on these previous reports and our clinical experience. As a result, the stress-related hormone levels in the present study were similar to those in these reports. Moreover, SC reflected each response of the hormones induced by the 3 drugs.

In particular, in the fentanyl treatment, SC significantly reflected the hormone responses. Fentanyl has a different mechanism of action from medetomidine and acepromazine, as it activates opioid receptors in the CNS, resulting in an increase of postsynaptic  $\text{K}^+$  extracellular outflow and a decrease of presynaptic  $\text{Ca}^{2+}$  intracellular inflow. This process inhibits neuron activation and neurotransmitter secretion, inducing sedation [4]. Moreover, opioids act at central opioid receptors to activate sympathetic outflow and

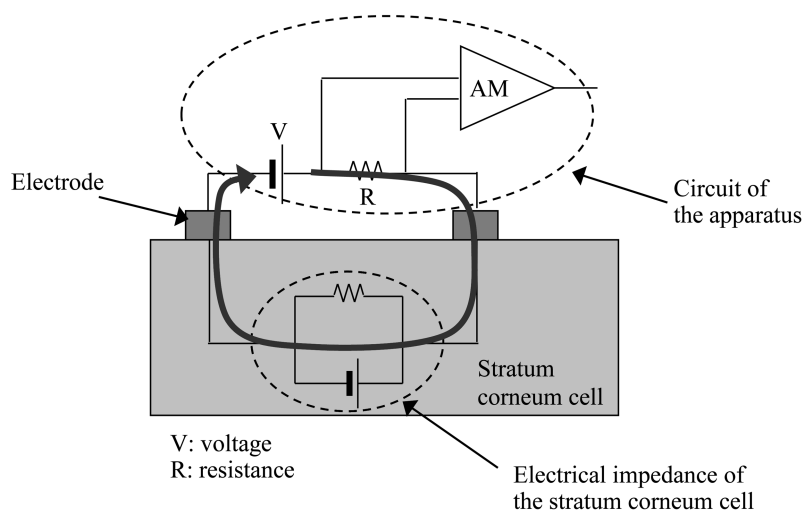


Fig. 1. The mechanism of the PS-IMP001<sup>®</sup>, the SC measurement instrument. SC is measured by passing a small amount of electrical current through electrodes that are applied to skin. Stress activates the SNS and sweat glands, which leads to increased SC.

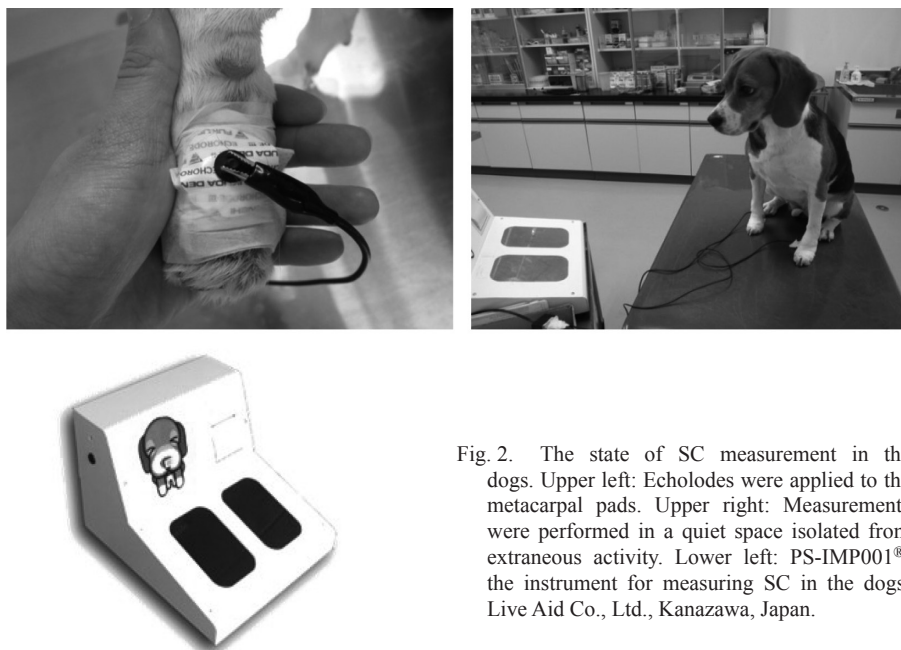


Fig. 2. The state of SC measurement in the dogs. Upper left: Echolodes were applied to the metacarpal pads. Upper right: Measurements were performed in a quiet space isolated from extraneous activity. Lower left: PS-IMP001<sup>®</sup>, the instrument for measuring SC in the dogs, Live Aid Co., Ltd., Kanazawa, Japan.

increase catecholamine secretion [9, 13]. In healthy male humans, increasing doses of fentanyl induced a significant dose-dependent increase in plasma catecholamine levels [9]. This process is not yet clearly understood, but it is suggested that opioid-induced catecholamine release is mediated by a particular type of opioid receptor in the CNS [15]. In the present study, both SC and catecholamine levels increased in the fentanyl treatment group. The limbic system in the brain is a CNS area in which the effects of stress are first visible. Yamamoto *et al.* [23] reported that when the central sympathetic system was destroyed by a neurotoxin in rats,

SC values decreased. Moreover, there were correlations between SC and 5-HT levels in the patients of depression, who have a disorder in 5-HT action in the CNS [14].

On the other hand, in the present study, the cortisol level increased in the fentanyl treatment group. Previous study had not reported the influence of fentanyl on the HPA axis or cortisol secretion. Moreover, there is no evidence of a relationship between SC and the HPA axis. It is thought that there may be problems in the measuring protocol for cortisol or that SC may reflect not only SNS activity in the SAM axis but also in the HPA axis. We would like to measure cortisol

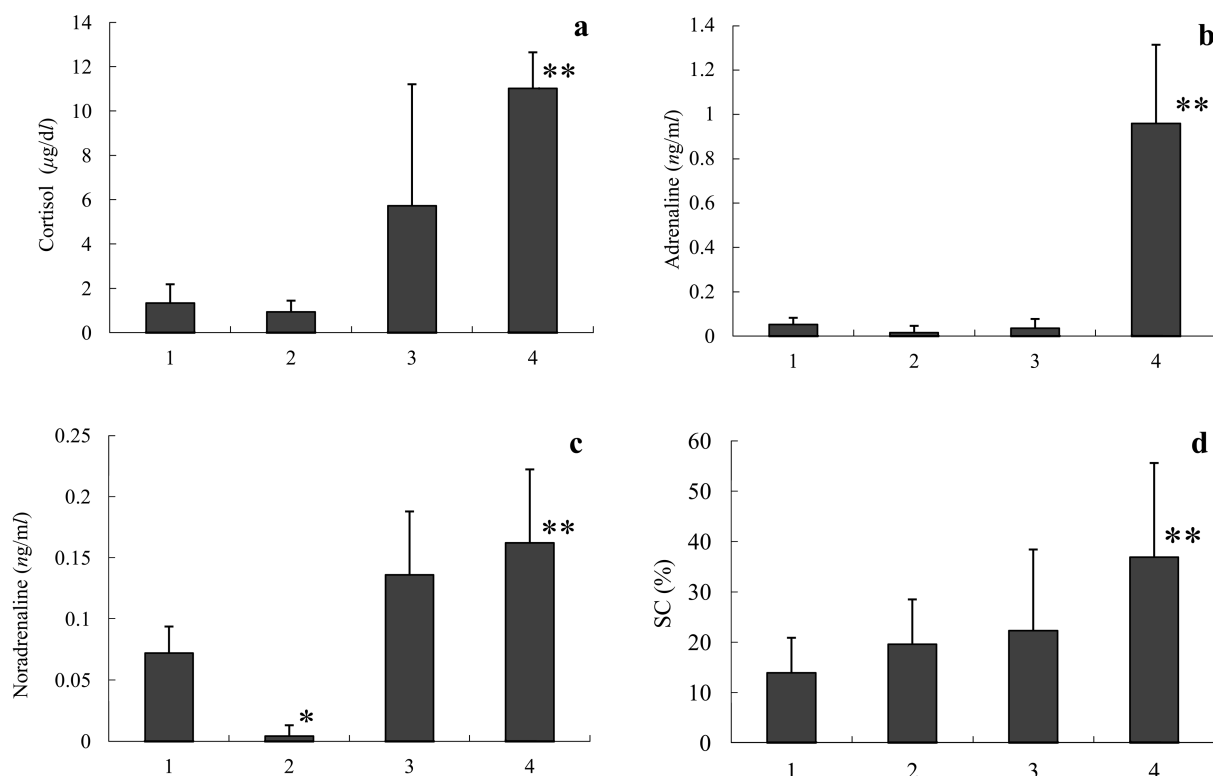


Fig. 3. Results of the Dunnett's test for a) cortisol, b) adrenaline, c) noradrenaline and d) SC. The graphs show the means (SD) of 5 dogs in all 4 treatments: 1) control, 2) medetomidine IM at 0.01 mg/kg, 3) acepromazine IM at 0.1 mg/kg and 4) fentanyl IV at 0.02 mg/kg. \*Significantly different versus the control group ( $P < 0.05$ ). \*\*Significantly different versus the control group ( $P < 0.01$ ).

levels in more cases and verify the relationships among SC, the HPA axis and cortisol secretion.

Consequently, it is considered that SC may be useful as an indicator of stress in dogs like stress-related hormones and that there may be some relationships between SC and SNS activity induced by CNS stimulation.

Further studies are necessary to more clearly define the relationships among SC, SNS activity and stress-related hormones. Moreover, whether SC actually can reflect varying degrees of stress in the manner to which stress-related hormones do when dogs are exposed to varying levels of stress still needs to be determined.

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