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Variation of Body Temperature after Administration of Amino Acid Amides

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ABSTRACT

Oxypinnatanine, a unique derivative of glutamic acid or glutamine with a furfuryl group increased the total time of NREM sleep by an oral administration. However the mechanism by which oxypinnatanine promotes sleep remains to be clarified. In this study, the effect of oxypinnatanine on heat release of freely moving mice from peripheral blood vessel was demonstrated by using thermography. Oxypinnatanine given at 30 mg kg^{-1} indicated the transient skin temperature elevation from 2 min after its administration but the skin temperature was decreased from 32 min after. Therefore, it is possible that the thermal release associated with the dilation of peripheral vessels is one of the mechanisms of oxypinnatanine about its promoting sleep. In addition, the effects of amino acid amides (30 mg kg^{-1}) which isolated from *Hemerocallis fulva* var. *sempervirens* was also examined. As a result, pinnatanine and kwansonine A decreased the temperature, while longitubanine A, longitubanine B and kwansonine B did not. As changes in body temperature are generally associated with concomitant changes in sleep propensity and the effects of sleep promoting substances are related to changes in thermoregulation. Therefore, it is possible that the monitoring on heart release from peripheral blood vessel with the use of thermography might enable the search for substances which induce sleep.

Key words: Oxypinnatanine, pinnatanine, kwansonine A, thermography

INTRODUCTION

The importance of sleep has recently been emphasized throughout the world as there are many people who have sleep problems in the modern world. For example, a meta-analysis has revealed that approximately 30% of the general population present with insomnia symptoms (Ohayon, 2002). Therefore the amelioration of insomnia is very important for society. There are many causes of insomnia such as poor physical condition, changing environment, mental stress and alcohol or medicine side effects, to name a few. Though there are several possible treatments according to the cause, one of the treatments, controlling body temperature, has been attracting attention recently. For example, many people who have poor blood circulation may have a sleeping problem or those who have menopausal troubles may not be able to sleep because of feeling very flushed. Sleep and body temperature is significantly correlated. The body temperature includes core body temperature and skin temperature and changes in core body temperature are generally interrelated with sleep (Zulley *et al*., 1984). The core body temperature drops with the onset of sleep, continues to decrease during sleep and gradually rises with the awakening from sleep (Czeisler *et al*., 1980; Zulley *et al*.,1984; Barrett *et al*., 1999; Foret *et al*., 1993). Therefore, changes of core body

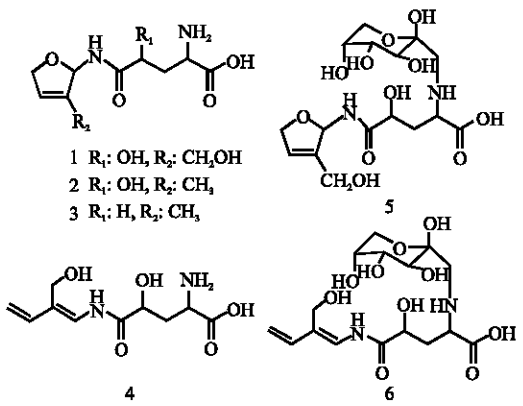

Fig. 1: Structures of isolated compounds from *Hemerocallis fulva* var. *sempervirens*

temperature mediate the changes in sleepiness. The core body temperature decreases because of the release of heat from a peripheral site and it develops the following process. (i) The peripheral blood vessel dilates and the heat transfer occurs as a result of increased blood flow (ii) the skin temperature rises with the transfer of heat (iii) the core body temperature and skin temperature decrease in conjunction with the increase of the amount of thermal release. Then, it might be possible to monitor this thermal release in sleep promoting stage by measuring of the skin temperature using thermography. Moreover, the monitoring of it might be useful when searching for substances that can induce sleep. Here, the thermal release in an initial stage of the sleep reaction was investigated by using a thermography.

In the continuing study for sleep promoting substances from natural resources, it was found that the flowers and leaves of *Hemerocallis fulva* L. var. *sempervirens* (Araki) M. Hotta (Liliaceae) are known to be taken as an aid for sleeping in Okinawa, Japan (Uezu, 1998). Previously, four known amino acid amides, oxypinnatanine (1), longitubanine A (2) and longitubanine B (3), pinnatanine (4) and two novel amino acid amides, kwansonine A (5) and kwansonine B (6), were isolated from the leaves of this plant (Ogawa and Konishi, 2009). These structures are shown in Fig. 1. Moreover, it was demonstrated by electroencephalographic analyses that an oral administration of oxypinnatanine (1) to mice increased non-rapid eye movement (non-REM, NREM) sleep in a dose-dependent manner (Ogawa *et al*., 2013). However, the mechanism by which oxypinnatanine promotes sleep remains to be clarified.

In the present study, the mechanism of oxypinnatanine (1)-induced sleep was investigated, using the monitoring of thermal release with dilatation of peripheral vessels. Furthermore, the effects of amino acid amides, which were isolated from *H. fulva* var. *sempervirens* were also investigated.

MATERIALS AND METHODS

Materials: Diazepam (cercine) (Qiu *et al*., 2009) and ramelteon (rozerem) (Miyamoto, 2006) were from Takeda Pharmaceutical Co., Ltd. Zolpidem (myslee) (Vinkers *et al*., 2010) was from Astellas Pharma Inc. Diphenhydramine (restamin kowa tablet) (Matsuoka and Kubota, 1897) was from Kowa Co., Ltd. These agents were dissolved in water. Glycine (Bannai and Kawai, 2012) was purchased from Nacalai Tesque; capsaicin (Kawabata *et al*., 2009) was from Wako Co., Ltd. Amino acid amides (compounds 1-6) were extracted and purified from *H. fulva* var. *sempervirens*.

Separation of these compounds was carried out according to the method described previously (Ogawa and Konishi, 2009).

Animals: Male ddY mice, 18-28 g (4-6 weeks old), were used in this study. They were housed at an ambient temperature of 24±2°C on an automatically controlled 12: 12 h light-dark cycle (light on at 08:00, illumination intensity of approximately 100 lux). The experimental protocols were approved by the Animal Care Committee of Doshisha Women's College of Liberal Arts.

Skin temperature measurement: Temperature were continuously monitored at 24±2°C. Each mouse was placed in an individualized cage for 90 min prior to the experiment and throughout the measurements. The skin temperature of mice was monitored with a calibrated IR camera (FLIR E60 Compact Thermal Imaging, 320×240 pixels IR Resolution). During IR recordings, both the mice and the camera were kept away from external IR sources and the distance between mice and the camera was 80 cm. The skin temperature of unanesthetized mice were tracked (n = 5). The normal skin temperature was measured for 30 min before the experiment. The temperature was measured for 80 min from administration of water or test substances. Average temperature on the back of mice was subsequently computed for every IR image using FLIR R and D software from FLIR Systems. All results were expressed as mean±SE of the difference of the normal skin temperature of each mouse due to large individual differences in skin temperatures.

Statistical analysis: Two-way analysis of variance (ANOVA) was used to test for statistical differences. When significant differences ($p<0.05$) were identified, the data were further analyzed by Student's t-test.

RESULTS AND DISCUSSION

The sleeping drugs: Diazepam (benzodiazepines group), zolpidem (not benzodiazepines group) and ramelteon (agonist of melatonin receptors) were used with the monitoring of the change of the skin temperature.

Diazepam is one of the most famous sleeping drugs and it has been the mainstay of therapy for insomnia and anxiety. Since diazepam was discovered in the 1950s, a large number of benzodiazepines analogs have been synthesized to obtain a superior risk/benefit ratio. Compared with the water group, diazepam at 12 mg kg^{-1} immediately and significantly decreased the skin temperature 2 min after administration by 0.7°C and 30 min after by 1.7°C (Fig. 2a). This result was consistent with the time when the mice were promoted into sleep.

Zolpidem is a widely used hypnotic drug which potentiates GABAergic neurotransmission by acting at the benzodiazepine site of $GABA_A$ receptors (Langer *et al.*, 1992). Zolpidem at 10 mg kg^{-1} decreased the skin temperature 0.5°C to 8 min and 1.3°C to 22 min after its administration, as compared with the water administrated group (Fig. 2b).

Ramelteon is a selective agonist to melatonin MT_1/MT_2 receptors in the suprachiasmatic nucleus; SCN (Kato *et al.*, 2005) and has circadian (Richardson *et al.*, 2008) and sedative effects (Roth *et al.*, 2005). When ramelteon at 10 mg kg^{-1} was administrated, the skin temperature was decreased 8 min after its administration statistically significantly and -1.0°C was indicated at 22 min as compared with the water group (Fig. 2c). These suggested that the skin temperature is markedly decreased by administration of sleeping drugs.

Diphenhydramine is an antagonist to histamine H_1 receptor and clinical use of H_1 receptor promotes sleepiness as a side effect (Nicholson *et al.*, 1985). Diphenhydramine given at 50 mg kg^{-1} significantly decreased the skin temperature by 1.5°C like sleeping drugs from 20 min (Fig. 2d).

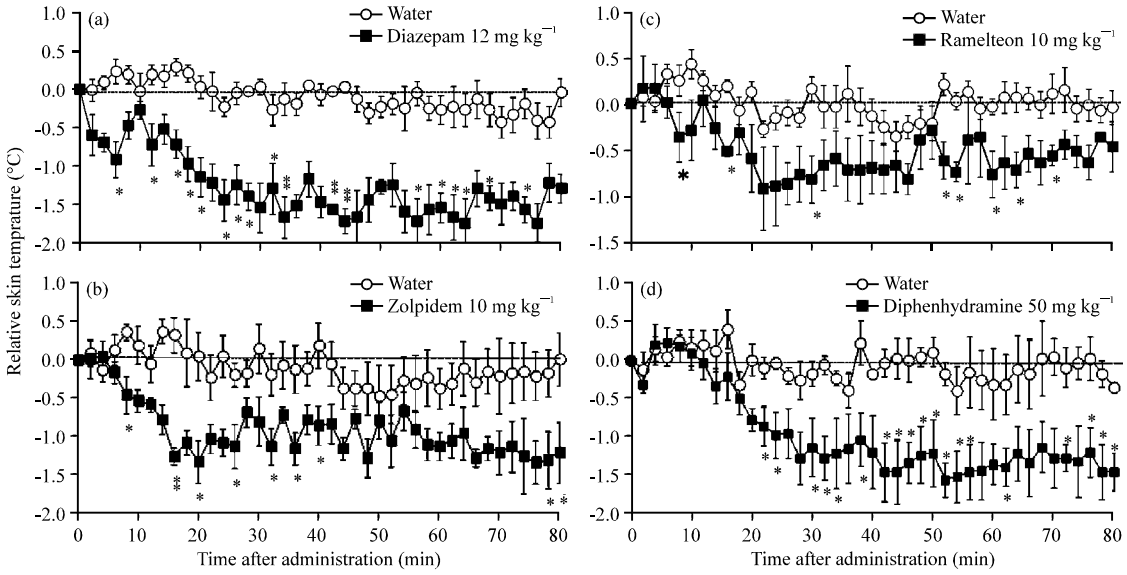

Fig. 2(a-d): The effect of sleeping drugs on the skin temperature of mice, administration group (n = 5). Each circle represents the mean±SE of the difference of the normal skin temperature of each mouse. *p<0.05, **p<0.01 versus water group by student's t-test after two-way analysis of variance was used to test for statistical differences (p<0.05)

Therefore, it is possible to monitor thermal releases in sleep promoting stage by measurement of the skin temperature using thermography. There have been many reports on the relationship of core body temperature or skin temperature and sleep but this is the first report using thermography. Then, this assay method was used about a sleep promoting substances.

Several natural compounds have been shown to improve insomnia in mice; for example glycine (Yamadera *et al*., 2007), verbenalin (Makino *et al*., 2009), crocin (Masaki *et al*., 2012) and ornithine (Omori *et al*., 2012) were reported to be effective in promoting NREM sleep. Especially, glycine (2 g kg^{-1}, p.o.) improve sleep by decreasing core body temperature with dilatation of peripheral vessels (Bannai and Kawai, 2012). Then the effect of glycine on the skin temperature was demonstrated. As a result, Glycine at a dose of 2 g kg^{-1} was risen the transient skin temperature elevation (+0.5°C) after its administration, because of dilatation of peripheral vessels and it was decreased (-1.2°C) from 30 min after its administration (Fig. 3a). This result was consistent with the literature data or the sleep-onset time (Brooks and Peever, 2011). Therefore it is possible to make a measurement of the transient temperature elevation with dilatation of peripheral vessels by using thermography measurement. Next, the effect of a substance which has a vasodilator action was demonstrated.

Capsaicin is the major ingredient in hot peppers of the plant genus *Capsicum*. It is used extensively in foods and is also utilized as a traditional medicine worldwide for the treatment of various disorders. Capsaicin (10 mg kg^{-1}, p.o.), which is known as a vasodilating agent (Salem and Dunbar, 2002) significantly elevated the skin temperature immediately after its administration; and the effect was statistically significant from 14 min after the administration (Fig 3b). However it did not decrease the body temperature compared with the water administration and it did not change the amount of sleep time (Surh *et al*., 1995). Thus, only vasodilating does not seem to induce sleep but it seems to be more important for the introduction of sleep through its decrease of body temperature with thermal release.

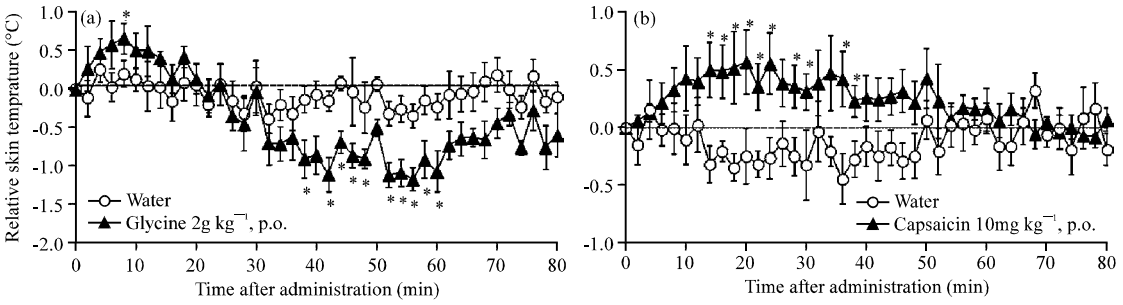

Fig. 3(a-b): The effect of glycine or capsaicin on the skin temperature of mice, administration group (n = 5). Each circle represents the mean±SE of the difference of the normal skin temperature of each mouse. *p<0.05 versus water group by student's t-test after two-way analysis of variance was used to test for statistical differences (p<0.05)

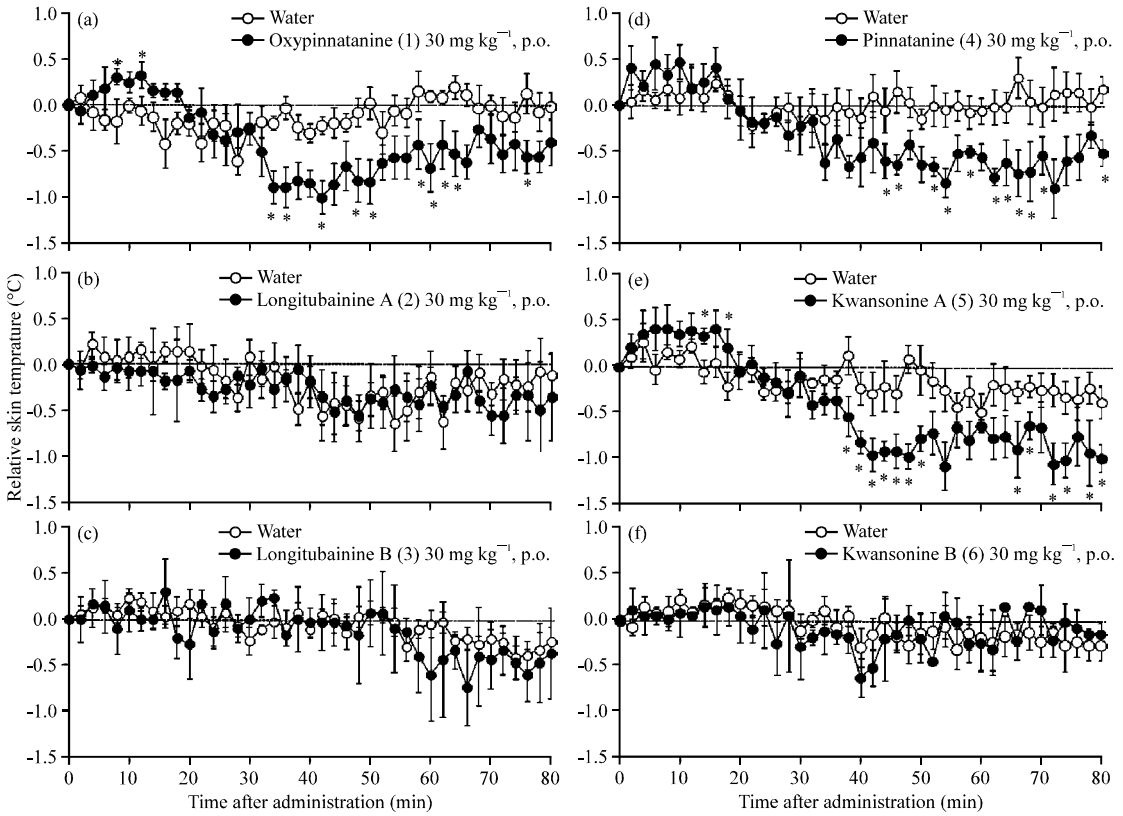

Fig. 4(a-f): The effect of amino acid amides (compounds 1-6) isolated from *H. fulva* var. *sempervirens*, administration groups (n = 5). Each circle represents the mean±SE of the difference of the normal skin temperature of each mouse (n = 5). *p<0.05 versus water group by student's t-test after two-way analysis of variance was used to test for statistical differences (p<0.05)

There the effect of oxypinnatanine (1) which was isolated from *H. fulva* var. *sempervirens* was examined. As a result, 1 given at 30 mg kg^{-1} indicated the transient skin temperature elevation (+0.3°C) after its administration but it was decreased (-1.0°C) from 32 min after its administration (Fig. 4a). Because this result showed the same tendency with glycine, it is suggested that the

thermal release associated with the dilation of peripheral vessels is enumerated in one of the mechanisms of 1 on promoting sleep. These results indicate that the monitoring system of skin temperature could be utilized as one of the assay methods to search for substances that promote the sleep.

The effects of compounds (2)-(6) which were isolated from *H. fulva* var. *sempervirens* were also examined. Compounds (2)-(4) have been isolated from only a few plants, such as *Euscaphis japonica* (Grove *et al*., 1973) and *Honkenya peploides* (Cerantola *et al*., 2005), besides the *Hemerocallis* species (Inoue *et al*., 1990). Moreover, there has been no study about the bioactivity of these compounds. In this study, 4 and 5 at a dose of 30 mg kg^{-1} showed the same tendency of glycine or 1. In contrast, compounds 2, 3 and 6 showed no effect on skin temperature of the mice (Fig. 4b-f). Therefore, it was suggested that compounds 4 or 5 might have a sleep promoting effect and, 2, 3 or 6 have no effect. Further study is being done on the sleep promoting activity of compounds (2)-(6).

CONCLUSION

In this study, it is indicated that the thermal release in sleep promoting stage can be monitored by using a thermography. Moreover, oxypinnatanine (1) indicated the transient skin temperature elevation after it's administration and that temperature was decreased after the transient elevation. Thus, the thermal release associated with the dilation of peripheral vessels is one of the mechanisms of 1 about its promoting sleep.

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