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Overseas Survey of the Effect of Cedrol on the Autonomic Nervous System in Three Countries

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Abstract To clarify the influences of ethnic and regional characteristics, and differences in perception on the cedrol effect on autonomic nerve activity, we compared women in their 20s–40s in Norway, Thailand, and Japan. A questionnaire survey of sense of stress and sleep conditions was performed at the same time. The degree of perceived stress, using a 30-item checklist, was highest in Japanese women. The mean stress score exceeded 5.0 in Japanese women, significantly higher than in Thai women ($p<0.05$) and Norwegian women ($p<0.01$). Sleeping time was shortest in Japanese women in all generations among the three countries. As the index of autonomic nervous activity, the miosis rate (ratio of pupil-diameter variation after light stimulus to initial pupil diameter) in pupillary light reflex was measured before and after cedrol inhalation. The miosis rate significantly increased after cedrol exposure compared to that before exposure in all three countries, suggesting that the parasympathetic nervous system became dominant. These findings suggested that cedrol produces a sedative effect in people of the three countries despite differences in the ethnic and living environments. *J Physiol Anthropol 26(3): 349–354, 2007* http://www.jstage.jst.go.jp/browse/jpa2
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Keywords: cedrol, cedarwood, the autonomic nervous system, parasympathetic nervous activities dominant

Introduction

Recently, odorous components derived from plants such as phytoncid, which smells of wood, and green leaf alcohol, which is similar to the odor of green leaves, have been reported to have sedative and stimulant effects, and have also attracted attention from a physiological viewpoint (Sugano et al., 1986). In general, however, our likes and dislikes and our sensitivity to odorous components vary, and their physiological effects may differ widely according to gender, age, race (Hans et al., 1999), and ethnic group, making scientific or statistic analysis difficult. It is therefore important to develop odorous components that produce such physiological effects regardless of the preference for or perception of their odor, and the physiological and psychological uses of odorous components can be expected to increase.

We have discovered by extensive physiological analyses that cedrol, an odorous component derived from cedarwood oil extracted from conifers, such as the Himalayan cedar and pine, acts on the autonomic nervous system, producing a sedative effect, such as significantly reducing the heart rate and systolic and diastolic blood pressures, by shifting the balance of autonomic activity to the parasympathetic side (Nagashima et al., 2002; Dayawansa et al., 2003; Kagawa et al., 2003). Furthermore, we examined the effect of cedrol on sleep in young Japanese women who complained of sleep disorders, and confirmed that cedrol produces favorable effects such as significantly shortening the sleep latency and prolonging the total sleep time (Yamamoto et al., 2003). It was of particular note in Japanese adults that these effects were hardly influenced by the degree of perception of, or preference for, the odor of cedrol, or by gender and age.

In this study, to clarify the effect of cedrol on the autonomic nervous system, the perception of its odor, including ethnicity and environment, we compared women living in three countries: Norwegian women living in a coniferous wood region, Thai women living in a tropical forest region, and Japanese women. If a sedative effect of cedrol can be confirmed in various countries as well as Japan, a universal approach to understanding its physiological effects, such as improvements in sleep quality, circadian rhythm, and stress level can be achieved. The subjects also completed a questionnaire concerning their perceived stress and sleep.

The results of this survey in three countries suggested that there are no regional or ethnic influences on the sedative effect of cedrol on the autonomic nervous system.

Methods

Subjects

One hundred and seventy-eight women in their 20's to 40's (Japanese: 64 (18 in their 20's, 22 in their 30's, and 24 in their

40's); Thai: 57 (19 in their 20's, 19 in their 30's, and 19 in their 40's); Norwegian: 57 (20 in their 20's, 18 in their 30's, and 19 in their 40's)), who lived in or near the capital cities of their countries, with jobs that did not involve night shifts or alternating shifts, who were not pregnant, who had no allergies, and who were non-smokers, were selected as subjects after appropriate advance explanation of the objectives and methods of the physiological assessments of this investigation and the obtaining of their consent. There were many conifers, including Himalayan cedars, in the Norwegian capital, Oslo, while there are no conifers in the Thai capital, Bangkok. For Thai women, individuals born in Bangkok who had never been to northeast regions of Thailand were selected. Based on these prior investigations and the selection of subjects, the Norwegian subjects had been surrounded by conifers since childhood, and the Thai subjects had never been exposed to conifers, and thus had never smelled cedrol scent. These surveys were carried out and assessed from April to June.

Questionnaire inquiry

The perceived state of stress was studied using a 30-item stress checklist (Oritsu et al., 1990). The perceived state of sleep was studied using a sleep check sheet (Yamamoto et al., 1999). These check sheets were translated from Japanese into Thai and Norwegian, and the expressions used and meaning of the questions were carefully evaluated in each country before they were used in the study.

Purity and presentation method of cedrol

Cedrol was purified from cedarwood oil extracted from the heartwood of Himalayan cedars, and a preparation with 99.9% purity (analyzed by gas chromatography) was used as the standard preparation in this study. For physiological evaluations, the cedrol odor was presented by vaporizing cedrol crystalline powder on a heater (amount of inhalation: $0.36 \pm 0.13\ \mu g/l$) and having the subjects inhale the vapor. The same standard preparation was used in all 3 countries.

Procedures of survey and physiological measurements

The investigation and physiological measurements were carried out at $24 \pm 1°C$ temperature and $55 \pm 5\%$ room humidity, and $225 + 25$ lx in the room. The subjects were acclimated to the experimental environment for 15 minutes before the test, and the test was performed in a sitting position. The light reflex of the pupil was measured based on the theory of using an instrument (KAO-PDMS-1developed by our company) to measure the pupil diameter (Ishikawa, 1986). After the initial pupil diameter was measured in a dark field, miosis was measured by presenting a light stimulus five times for 0.25 seconds. The rate of the pupil diameter at maximum miosis after light stimulation relative to the initial pupil diameter (miosis rate) was calculated as a parameter of the autonomic nervous system state (Utsumi et al., 1978). The subjects then inhaled cedrol vaporized on a heater-vaporizer (6 mg/hr) for 1 minute. Similar light stimulation was performed during cedrol inhalation, and miosis was measured. The miosis rate was compared between before (pre-) and after (post-) cedrol inhalation, and the state of the autonomic nervous system was evaluated (Hasegawa and Ishikawa, 1989; Miyazaki, 1991; Shirakawa, 1995).

Evaluation of the perception and preference for cedrol

After measuring the pupil diameter, the subject held a tube containing powdered cedrol crystals under the nose and smelled for 20 seconds, and subjects who perceived the aroma were asked about their taste for the aroma.

Statistical analysis

All statistical analyses were carried out using the package software Stat View 5.0 for Windows. One-factor factorial ANOVA was used to investigate the stress score (Fig. 1-1 and 1-2) and total sleeping time (Fig. 2) in the three countries. For analysis of the miosis rate, a paired t-test was used (Fig. 3). For the relationship between the stress score based on the 30-item checklist and the measurement of pre-miosis rate five times among the three countries, one-factor factorial ANOVA was used (Fig. 4). For investigation of the relationship between the ratio of subjects who could perceive the cedrol scent and the measurement of post-miosis rate five times, a Chi-square test was used (Fig. 5).

Results

In this survey, the sense of stress of the adult females in the three countries was analyzed in the inventories. Figures 1-1 and 1-2 show the stress survey results using a 30-item stress checklist. The degree of perceived stress was highest in Japanese women, significantly higher than in Thai women ($p<0.05$) and Norwegian women ($p<0.01$). The mean stress score exceeded 5.0 in Japanese women (Fig. 1-1). Stress was perceived most by Japanese women in each age group, and significantly greater stress was perceived by Japanese women than Norwegian women (20 s: $p<0.01$, 30s: $p<0.05$, 40s: $p<0.05$) in all age groups (Fig. 1-2).

The duration of sleep decreased gradually with increased age in all three countries; particularly, total sleeping time was shortest in Japanese women (mean 399 minutes) in all generations among the three countries, and was significantly shorter than Thai women (20s: $p<0.01$, 40s: $p<0.01$) and Norwegian women (30s: $p<0.05$), as shown in Fig. 2. Comparison of total sleeping time, waking up, and initiating sleep among the three countries was presented. The data were analyzed statistically, but a significant difference was not obtained except for total sleeping time.

In this survey, the effect of cedrol on the autonomic nervous system was investigated by measuring the change rate of miosis in reaction to light as the index. When the miosis rate determined by the pupil light reflex experiment was compared between before (pre-) and after (post-) cedrol presentation, it increased significantly after cedrol inhalation in the three

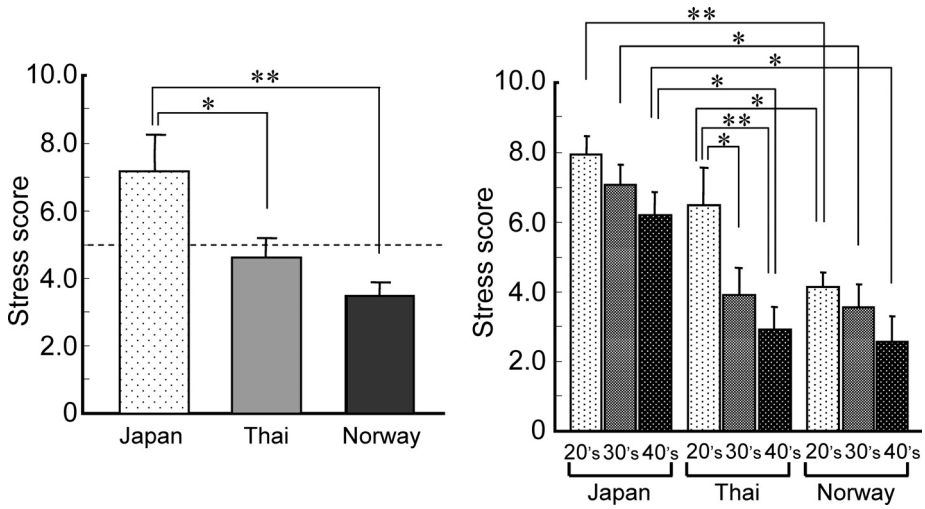

Fig. 1 (1) Comparison of awareness of stress among the three countries. (*: $p<0.05$, **: $p<0.01$, mean±SE), (2) Comparison of awareness of stress among age groups (*: $p<0.05$, **: $p<0.01$, mean±SE).

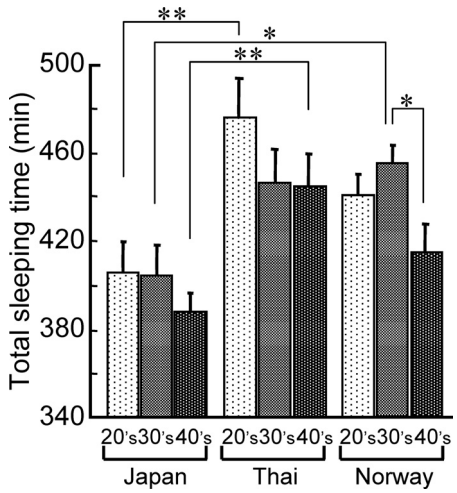

Fig. 2 Comparison of awareness of total sleeping time among the three countries. (*: $p<0.05$, **: $p<0.01$, mean±SE).

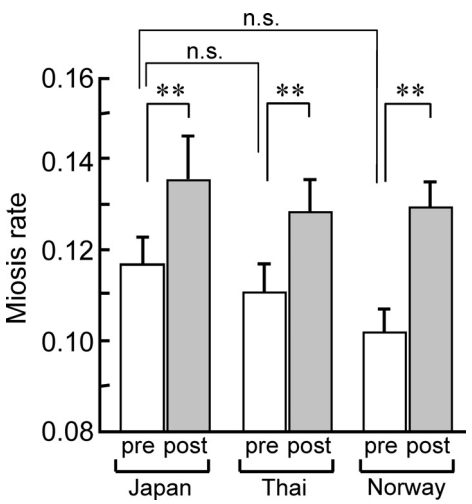

Fig. 3 Comparison of awareness of miosis rate among the three countries. (**: $p<0.01$, mean±SE, n.s.=not significant. mean±SE).

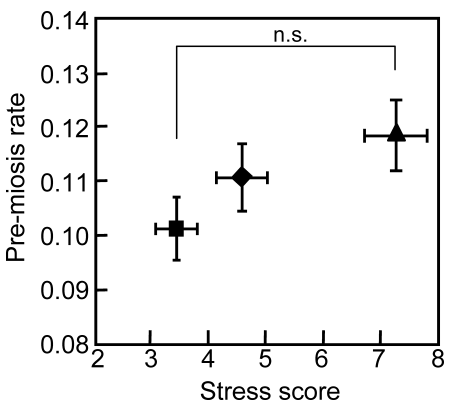

Fig. 4 Relationship between pre-myosis rate and stress score. (n.s.=not significant. mean±SE).

countries, as shown in Fig. 3.

We further compared the relationship between the stress score based on the 30-item stress checklist and the pre-miosis rate before cedrol presentation among the three countries. The results are shown in Fig. 4. The pre-miosis rate was 0.118 in the Japanese, who showed the highest score on the 30-item stress checklist, i.e., they perceived the strongest stress (mean stress score 7.3), 0.112 in Thais (mean stress score 4.6) and 0.101 in Norwegians (mean stress score 3.4). The tendency of miosis rate increase was shown as increasing stress. One-factor factorial ANOVA was performed, but a significant difference was not obtained ($p=0.0772$).

We evaluated the effect of cedrol on the autonomic nervous system based on the miosis rate according to whether the subject perceived the odor of cedrol during inhalation (Fig. 5). The percentage of subjects who recognized the odor of cedrol was highest in Norway (79%), followed by Japan (73%), and then Thailand (63%), and the recognition of the odor in Norwegian women was significantly higher than in Thai women ($p<0.05$). In Fig. 5, "Effect" means the significant

increase in the miosis rate after (post-) cedrol inhalation; on the other hand, "No effect" means no significant increase in the miosis rate. The percentages of subjects in whom parasympathetic activities based on the pupil reflex (analysis of the miosis rate) were dominant among subjects who perceived the cedrol odor were 91%, 79%, and 69% in Norway, Japan, and Thailand, respectively. The percentages of parasympathetic activities in Norwegian women were significantly higher than in Thai women ($p<0.05$), parallel to the recognition rate of cedrol odor. In those who did not recognize the cedrol odor, however, the percentage of subjects who showed a shift to a parasympathetic-dominant state scarcely differed among the three countries, being about 75% (75% in Norway, 71% in Japan, 76% in Thailand), but no significant difference was obtained.

Discussion

In the survey of stress awareness in three countries, the stress score was high in all generations of Japanese females, 5.0 or higher, suggesting that they were in a mild stress state (Oritsu et al., 1990). In Thai females, stress awareness in females in their 20s was as high as that in the same generation of Japanese females, and stress awareness may increase further in the young population of rapidly developing Bangkok. Periodic surveys and comparative surveys between cities and local areas may be necessary in the near future.

The survey of sleep conditions suggested that the sleeping hours tended to be short in the Japanese (Murakami et al., 1989). Since this survey was performed between April and June, the influence of the midnight sun should be considered in Norway, and it is necessary to carry out a survey in another season for comparison.

We therefore examined whether there was a correlation between perceived stress and the sleeping time in the three countries. Although perceived stress tended to increase inversely with sleep duration in Japan and Thailand, the relationship was not significant, and no correlation was observed in Norway. A clear correlation between perceived stress and sleep is considered to have been absent in this study, because the subjects of all three countries were healthy adult women, who were not considered to have been exposed to severe stress and had relatively good sleeping patterns.

We further evaluated whether there were differences in the perception rate of cedrol and its effects on the autonomic nervous system among the subjects. The measurement of the reaction of pupil light reflex has been widely used as a good method of autonomic nervous response analysis, particularly in the ophthalmology field. We employed this method because it is simpler than electrocardiography and the continuous measurement of blood pressure and is as reliable as measuring autonomic nervous activity, reducing tension and stress in the subjects during measurement.

First, the miosis rate before cedrol inhalation was measured using this method, and its correlation with stress awareness was investigated in the three countries. The miosis rate tended to increase as stress awareness increased (Japan>Thai>Norway); however, it is not currently clear whether this tendency indicates a correlation between autonomic nervous system response and mental conditions such as stress awareness. The subjects who participated in this survey were healthy and did not have strong stress awareness, and more extensive surveys and analysis, including subjects with stronger stress awareness, are necessary.

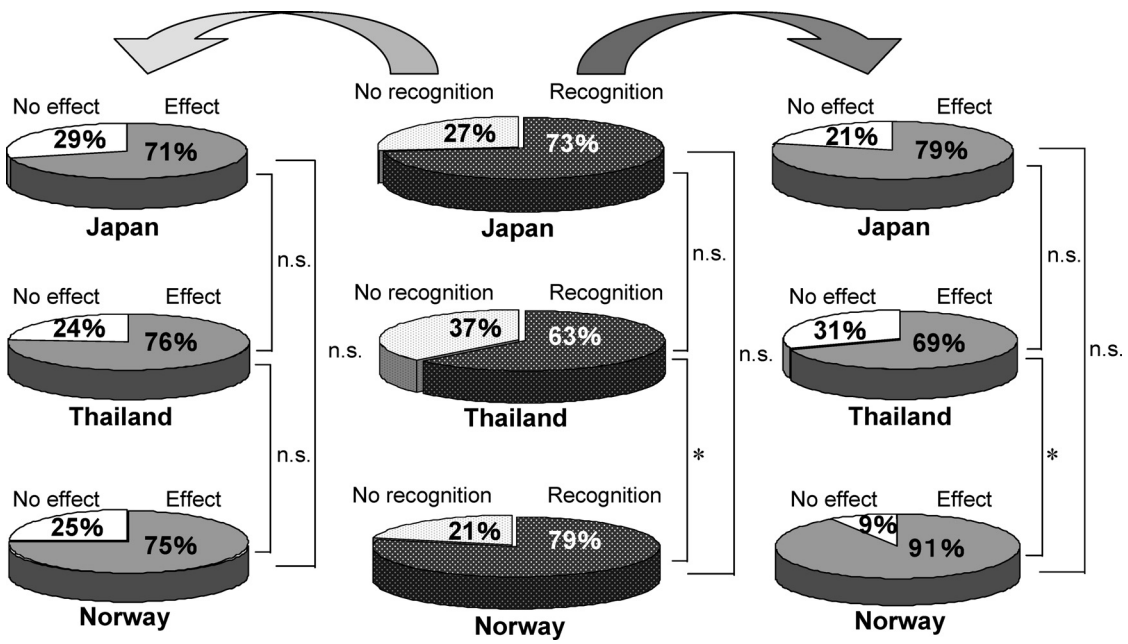

Fig. 5 Comparison of the recognition for cedrol odor and the effect in the miosis rate among the three countries. (*: $p<0.05$, mean±SE, n.s.=not significant. mean±SE)

Next, the effect of cedrol on the miosis rate was investigated.

In this study, although lifestyle and environment markedly differed among the three countries, cedrol inhalation significantly increased the miosis rate, confirming that cedrol enhances the parasympathetic nervous system (Dayawansa and Umeno, 2003). The high perception rate in Norwegians may be explained by their environment, in which cedrol is always emitted from the abundant conifers. This point needs further verification by comparison with other countries. This finding is also of interest in the conventional concept of the action mechanism of odorous agents.

Generally, the degree of preference and recognition of the odor component markedly affected the exhibition of physiological components (sedation or awakening), and the effects varied due to differences in sex (Doty et al., 1985; Ayabe-Kanamura et al., 1997) and age (Murphy et al., 1991), as well as lifestyle among races (Wysocki et al., 1991).

However, the sedative effect of the cedrol odor did not vary despite differences among the living environments and ethnics. Recognition of the cedrol odor differed among the three countries: Since there are many coniferous forests releasing cedrol in Norway, this living environment might lead to a high recognition rate, followed by the Japanese women, with the reaction (increase in the miosis rate) being lowest in the Thai women, who showed the lowest perception of the scent. In all three countries, about 70–75% of subjects who could not perceive cedrol odor reacted, showing no major difference among these countries. This requires verification by comparative surveys in other countries. These results are very important for consideration of the action mechanism of the sedative effect of cedrol, and then shows the interesting effects of odor in re-evaluating the current thinking that recognition of the aroma component and preferences are important in autonomic nerve system effects via the central nervous system. Although it is not known why such sedative effects occur without aroma recognition, Kagawa et al. (2003) recently performed an extensive study in laboratory animals and suggested that the sedative effect of cedrol is mediated via the pathway from the pharynx to the trachea, that is, the response of the autonomic nervous system via the vagus nerve of the lower respiratory tract and feedback to the central nervous system, not the general pathway via the upper respiratory tract system: olfactory cells in the nose→olfactory nerve→central nervous system. These findings suggest that not only the upper airway but also the lower airway reacts to cedrol in humans. The presence of the two reaction pathways may be the reason for the sedative effect noted in many subjects who could not perceive cedrol odor, and the higher frequency of the sedative effect in subjects who could perceive cedrol odor.

In the near future, we are planning to investigate the action mechanism of cedrol as well as its relationship with this index of stress evaluation and its effect on the autonomic nerve system from a global perspective.

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