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Effect of lavender aroma on salivary endocrinological stress markers

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ABSTRACT

Objective: We evaluated the stress relief effect of lavender aroma by measuring sensitive salivary endocrinological stress markers, cortisol and chromogranin A (CgA).

Design: Thirty healthy students performed a serial arithmetic task for 10 min and then rested for 10 min. During the resting period, 16 students (aroma group) were exposed to airborne organic essential oil of lavender. Saliva samples were collected immediately before and after the arithmetic task, and at 5 and 10 min after that. Salivary cortisol and CgA levels were determined by enzyme-linked immunosorbent assay.

Results: In the aroma group, levels of CgA that had been elevated at the end of the arithmetic task were statistically significantly lower 10 min later. The control group showed no such change. During the protocol, no statistically significant changes in levels of cortisol were detected in either the aroma group or the control group.

Conclusions: These findings suggest that lavender aroma has a stress relief effect.

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1. Introduction

Since ancient times, folk medicine has presented empirical evidence that aromatherapy with essential oils may have physiological and psychological effects. Lavender aroma has particularly been associated with mood enhancement.¹ Accompanying the recent development of complementary and alternative medicine, scientific investigation of the effects of aromatherapy have been undertaken. Several studies have suggested that lavender aroma may be associated with improved mood, reduced anxiety or mental stress, sedation, and good sleep.^{2–6} In the published literature, however, we have found little endocrinological evidence of the effects of aromatherapy. Consequently, in the present study, after exposing a group of people who were stressed by an arithmetic task to lavender aroma, we evaluated the stress relief effect by

measuring sensitive salivary endocrinological stress markers, cortisol and chromogranin A (CgA).

Endocrinological stress markers are useful for objectively evaluating stress. Their presence in saliva samples can be assayed, and the collection of saliva is a noninvasive, relatively nonstressful, and therefore highly convenient sampling method.⁷ Produced in the adrenal cortex, cortisol is the main glucocorticoid hormone in humans. It is released in response to various psychosocial stimuli via the hypothalamus–pituitary–adrenal (HPA) axis. The level of cortisol in saliva accurately reflects the level of active, free cortisol in the blood.^{7,8} CgA is an acidic glucoprotein that is released along with catecholamines from the adrenal medulla and the sympathetic nerve endings.^{9–11} According to a recent report, CgA is produced by human submandibular glands and secreted into saliva.¹² Salivary CgA has been receiving attention as a novel stress marker.^{13,14}

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2. Materials and methods

For the study, approved by the Ethics Committee of the Osaka University, we enrolled 30 healthy student volunteers (23 males, 7 females) aged 21–26 years old. None reported that they were receiving any medication. Prior to the study, informed consent was received, in writing, from each participant. They were randomly assigned to two groups: an aroma group (13 males, 3 females) and a control group (10 males, 4 females). Each protocol was conducted on different days to avoid aroma contamination of the control group. Both the groups spent 10 min taking the Uchida-Kraepelin test, a serial arithmetic task, for 10 min.^{15,16} Several psychophysiological studies have used this test as a mental stressor.^{17–19} A grid of 17 rows of random numbers from 3 to 9 is presented and subjects are required to add adjacent numbers horizontally across the rows as fast and as accurately as possible. After the arithmetic task, all the subjects rested for 10 min, during which time the aroma group, at a comfortable distance (approximately 10 cm from the nose), were exposed to airborne organic essential oil of lavender (Rohto Pharmaceutical Co. Ltd., Osaka, Japan) that had been infiltrated into filter paper (150 μ L). There was no air current in the room. Saliva samples were collected immediately before and after the arithmetic task, and at 5 and 10 min after that (Fig. 1). At the same time, subjective stress was measured using a 10-division visual analog scale, a 10 cm horizontal line with the right-hand end labeled “The worst stress I can imagine” and the left-hand end “No stress at all”. The participants were asked to mark the line according to their level of perceived stress. To minimize the effects of food and drink on the levels of salivary stress markers, participants were asked to abstain for food or drink, other than mineral water, for 2 h before saliva sampling.²⁰ Furthermore, to minimize the effects of circadian variation, the protocol took place in the afternoon.²¹

Saliva samples were collected using the Salivette system (Sarstedt Co. Ltd., Nümbrecht, Germany). This device extracts saliva samples by centrifuging (at 3000 rpm for 15 min) the cotton wads that subjects held in their mouths (for 2 min). During collection, the cotton wad was rolled around like a hard candy in the oral cavity. Consequently, the saliva samples were whole saliva composed of parotid, submandibular, and sublingual secretions. Moreover, in the Salivette system, the tactile stimulation of the presence of the cotton wad in the oral cavity tends to stimulate a rather uniform salivary flow.²² The samples were labeled and stored at -80°C until the assay. Levels of salivary cortisol and CgA were evaluated, according to a previously described method,^{23,24} with commercial enzyme-linked immunoassay kits: Enzaplate cortisol kit (Bayer Medical Ltd., Tokyo, Japan) and the Human CgA EIA kit (Yanaihara Institute Inc., Shizuoka, Japan). Levels of

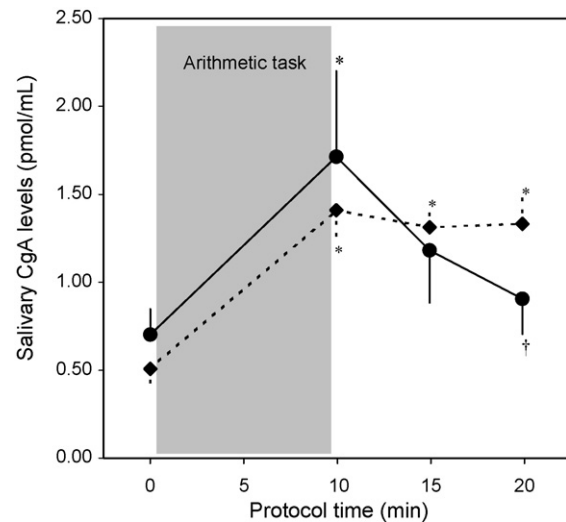


Fig. 2 – Changes during the experimental period in mean values (\pm S.E.) for salivary CgA (chromogranin A) levels in the aroma group (solid line, $n = 16$) and the control group (dashed line, $n = 14$). Statistically significant difference compared with 0 min and †10 min; $p < 0.05$ (repeated measures ANOVA and Bonferroni adjustment).

salivary cortisol and CgA were displayed as mean values \pm standard error. Student's t-test was performed to detect intergroup differences, and ANOVA with repeated measures to detect time-related differences. Meanwhile, to avoid ceiling/floor effects, scores for the visual analog scale were analyzed using non-parametric tests (Mann-Whitney U test and Friedman test) and were displayed as medians and quartiles. Bonferroni adjustments were used for all multiple comparisons. Values were considered to be significantly different when $p < 0.05$.

3. Results

In samples taken immediately after the arithmetic task (protocol 10 min), in both the aroma and the control group, we detected statistically significantly higher levels of salivary CgA than in those taken before the task (protocol 0 min): aroma group, 0.70 ± 0.15 pmol/mL vs. 1.71 ± 0.49 pmol/mL, $p < 0.05$; control, 0.51 ± 0.10 pmol/mL vs. 1.41 ± 0.17 pmol/mL, $p < 0.05$ (Fig. 2). In the aroma group, levels of CgA were statistically significantly lower in samples taken 10 min after the task (protocol 20 min) than in those taken immediately after (1.71 ± 0.49 pmol/mL vs. 0.91 ± 0.20 pmol/mL, $p < 0.05$). Meanwhile, in samples taken from the control group 10 min

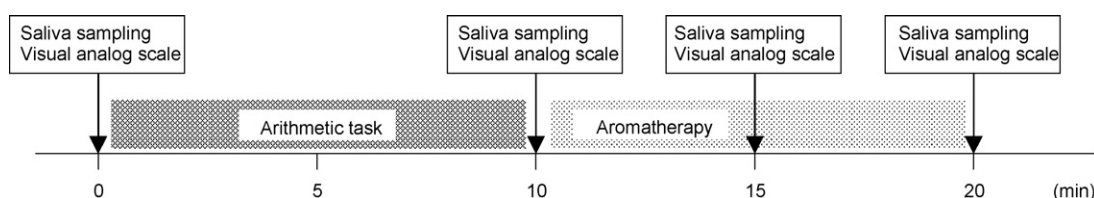


Fig. 1 – Study protocol.

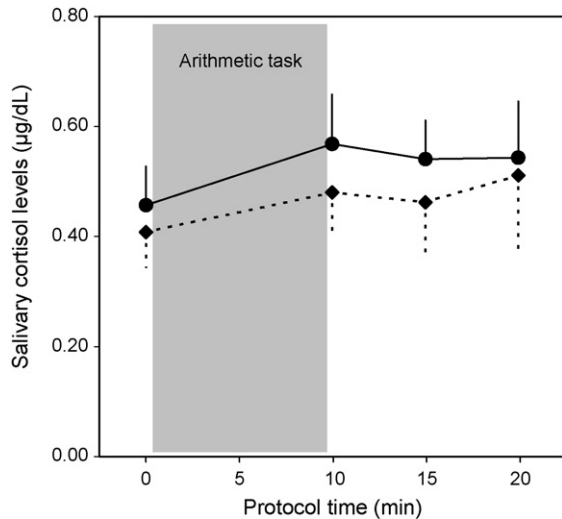


Fig. 3 – Changes during the experimental period in mean values (\pm S.E.) for salivary cortisol levels in the aroma group (solid line, $n = 16$) and the control group (dashed line, $n = 14$).

after the task, still higher levels of CgA were detected than in those taken before the task (0.51 ± 0.10 pmol/mL vs. 1.33 ± 0.15 pmol/mL, $p < 0.05$). In samples taken 5 min (protocol 15 min) or 10 min after the task, however, there was no significant difference in salivary CgA levels between the

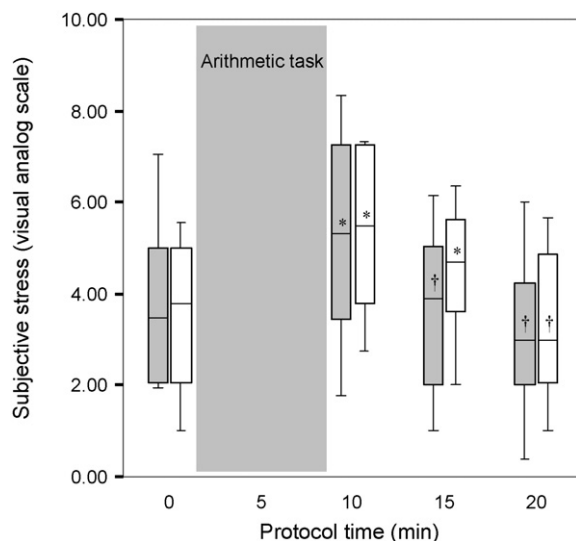


Fig. 4 – Changes during the experimental period in subjective perception of stress, evaluated using a visual analog scale, in the aroma group (gray box, $n = 16$) and the control group (non-shaded box, $n = 14$). In the boxplot, the line inside of the box represents the median value. The lower and upper regions of the box represent the lower and upper quartiles, respectively. The lower and upper horizontal lines outside of the box represent the smallest and largest observations, respectively. Statistically significant difference compared with *0 min and †10 min; $p < 0.05$ (Friedman test and Bonferroni adjustment).

aroma and the control group (1.18 ± 0.30 pmol/mL vs. 1.31 ± 0.10 pmol/mL, $p = 0.69$, and 0.91 ± 0.20 pmol/mL vs. 1.33 ± 0.15 pmol/mL, $p = 0.11$, respectively). During the experimental period, neither the aroma nor the control group showed any significant variation in the levels of salivary cortisol (Fig. 3).

Immediately after the arithmetic task (protocol 10 min), in both the aroma and the control group, subjective perceptions of stress, evaluated using a visual analog scale, were statistically significantly higher ($p < 0.05$) than before the task (protocol 0 min): aroma group, 3.5 (2.0 and 5.0) vs. 5.3 (3.3 and 7.5); control, 3.8 (2.0 and 5.0) vs. 5.5 (3.8 and 7.3) (Fig. 4). At 5 min after the task (protocol 15 min), the aroma group reported statistically significantly ($p < 0.05$) decreased subjective stress compared to immediately after the task: 5.3 (3.3 and 7.5) vs. 3.9 (2.0 and 5.0). Meanwhile, not until at 10 min after the task (protocol 20 min) did the control group report statistically significantly decreased ($p < 0.05$) subjective stress compared to immediately after the task: 5.5 (3.8 and 7.3) vs. 3.0 (2.0 and 5.0). At 5 min or 10 min after the task, there was no statistically significant difference in subjective stress between the aroma and the control group: aroma group 3.9 (2.0 and 5.0) vs. 4.7 (3.6 and 5.7) ($p = 0.12$); and 3.0 (2.0 and 5.0) vs. 3.0 (2.0 and 5.0) ($p = 0.40$).

No gender differences in the observed responses in salivary markers or subjective stress were apparent.

4. Discussion

In both the aroma and the control group, measured levels of salivary CgA immediately after the arithmetic task were statistically significantly higher than immediately before it. Similarly, for both groups, subjective perception of stress, evaluated using a visual analog scale, was statistically significantly higher. These findings add to the evidence that CgA levels may be a good index of mental stress. Previous studies have also found that salivary CgA levels are statistically significantly increased by the mental stress caused by things such as public speaking, word processing tasks, or driving a car on an expressway.^{13,14} Salivary CgA originates in the submandibular gland and, in response to activation of the autonomic nervous system innervating the submandibular gland, is released directly from the exocrine cells of the granular convoluted tubules into the saliva.^{12,25} Thus, the secretory mechanism is different from that of plasma CgA, which is released from the adrenal medulla and the sympathetic nerve endings.⁹⁻¹¹ In fact, a previous study found no significant changes in levels of plasma CgA associated with a different arithmetic task,²⁶ although salivary CgA was not assayed in that study. In our protocol, cortisol analysis revealed no significant variation in the levels of salivary cortisol in samples from the aroma group and the control group. The studies cited earlier report that increases in the level of cortisol attributable to mental stress occur considerably later than increases in CgA^{13,14} and increases in cortisol were unlikely to have occurred during the experimental period.

This time lag suggests that salivary CgA and cortisol may indicate different kinds of response to stress stimuli. Indeed,

the endocrinological stress response is known to exhibit two stages. The initial response, occurring within seconds, involves enhanced secretion of catecholamines from the sympathetic nervous system, hypothalamic release of corticotropin-releasing hormone into the portal circulation, and enhanced secretion of pituitary adrenocorticotrophic hormone. Steroid hormones are secreted more slowly.²⁷ Salivary cortisol, which appears long after initial stress, is not for a good marker for evaluating acute stress.

Ten minutes after the stress stimulus ended, levels of CgA that had been elevated in response to the arithmetic task statistically significantly decreased in the aroma group, but not in the control group. Previous studies have suggested that lavender aroma may suppress the activity of the sympathetic nervous system.^{28,29} Volatile compounds may enter the bloodstream by way of the nasal or lung mucosa, or may diffuse directly into the olfactory nerve and pass up to the limbic system which affects the sympathetic nervous system. Even so, in samples taken after the task, there was no significant difference in salivary CgA levels between the aroma and the control group. This may have resulted from closing the sampling 10 min after the task (protocol 20 min). Namely, the difference between both groups may have increased further after that. More extended sampling period is needed for future studies. Meanwhile, stress has both positive and negative aspects: eu-stress results in feeling uplifted or fulfilled, and negative di-stress results in feeling harried, or worse, when it is difficult to cope with a situation.³⁰ In recent studies, we found that moderate stress (eu-stress) such as laughter, spa bathing, or coffee intake is associated with significant increases in the levels of salivary CgA.³¹⁻³³ These results, however, are not inconsistent with the present findings. There may be a mechanism that triggers moderate sympathetic activity. Briefly, under normal conditions, eu-stress stimulates moderate sympathetic activity that is perceived as feeling pleasantly uplifted. On the other hand, under high-stress conditions in which sympathetic activity seems to reach its limits, eu-stress may restrain excessive sympathetic activity. In our studies of the effects of laughter or spa bathing, we found that increased levels of CgA associated with these stimuli were found only in people who had reported low initial levels of stress.^{31,32} People who had high levels of stress at the start of the protocol tended to show decreased salivary CgA levels after spa bathing. Furthermore, increased CgA levels after coffee intake were found in non-pregnant women, but were not found in pregnant women, who scored high for stress.³³ Now, in the present study, we have found that increased subjective stress caused by the arithmetic task decreased significantly 5 min later in the aroma group and 10 min later in the control group. This finding may be evidence that changes in mood might precede endocrinological changes.

The present findings suggest that lavender aroma can have a stress relief effect. Consequently, aromatherapy may be clinically useful for treating mental stress. In previous studies, besides lavender, the effects of aromatherapy using essential oils such as rosemary or peppermint have been investigated.^{3,6,34} To establish which aroma has the greater effect on mental stress, it is necessary to carry out further studies with various kinds of aroma.

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