

## Effects of Fragrance Inhalation on Sympathetic Activity in Normal Adults

Shinichiro Haze\*, Keiko Sakai and Yoko Gozu

*Product Development Center, Shiseido Co., Ltd., 2-2-1, Hayabuchi, Tsuzuki-ku, Yokohama 224-8558, Japan*

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**ABSTRACT**—We investigated the effects of fragrance inhalation on sympathetic activity in normal adult subjects using both power spectral analysis of blood pressure fluctuations and measurement of plasma catecholamine levels. Fragrance inhalation of essential oils, such as pepper oil, estragon oil, fennel oil or grapefruit oil, resulted in 1.5- to 2.5-fold increase in relative sympathetic activity, representing low frequency amplitude of systolic blood pressure (SBP-LF amplitude), compared with inhalation of an odorless solvent, triethyl citrate ( $P < 0.05$ , each). In contrast, fragrance inhalation of rose oil or patchouli oil caused a 40% decrease in relative sympathetic activity ( $P < 0.01$ , each). Fragrance inhalation of pepper oil induced a 1.7-fold increase in plasma adrenaline concentration compared with the resting state ( $P = 0.06$ ), while fragrance inhalation of rose oil caused a 30% decrease in adrenaline concentration ( $P < 0.01$ ). Our results indicate that fragrance inhalation of essential oils may modulate sympathetic activity in normal adults.

**Keywords:** Sympathetic nerve function, Power spectral analysis, Catecholamine, Essential oil, Fragrance inhalation

It has been reported that olfactory stimulation by fragrance inhalation exerts various physiological effects on humans. Effects of fragrance on brain function have been studied by using alpha and theta activity in the electroencephalogram (EEG) (1) or contingent negative variation (CNV) (2, 3). These studies have shown that some fragrances exert stimulant or inhibitory effects on brain function. Moreover, it has been reported that human endocrine and immune systems are affected by fragrance in a study that assessed the effects of fragrance on both endocrine function, by analyzing urinary cortisol and dopamine levels, and immune function, by analyzing natural killer cell activity and CD4/8 values (4).

On the other hand, effects of fragrance on the autonomic nervous system have been studied by noninvasive recording of various autonomic parameters such as heart rate (HR), skin conductance and respiration (5), or skin temperature, skin conductance, breathing rate, pulse rate and blood pressure (6). To our knowledge, there is no study that has been designed to quantitate the separate effects of fragrances on sympathetic and parasympathetic activities.

Power spectral analysis of heart rate variability or blood pressure fluctuations have been developed for the noninva-

sive assessment of autonomic nerve activity and utilized to assess the autonomic nerve activity in various disorders such as hypertension and diabetes mellitus (7–9). In such analyses, it is assumed that the low frequency components (LF) of blood pressure fluctuations and the high frequency components (HF) of heart rate variability reflect sympathetic activity and parasympathetic activity, respectively (10). In general, fast Fourier transform processing has been employed for power spectral analysis of fluctuations, a new method, which provides real-time, noise-adjusted calculation of sympathetic and parasympathetic activities by use of wavelet transform processing, has been developed (11).

We are interested in the effect of fragrance inhalation on human sympathetic activity and have an idea that fragrance inhalation may be utilized to correct sympathetic dysfunction associated with various life-style related disorders such as hypertension and obesity. To examine this possibility, we investigated the effect of fragrance inhalation on sympathetic activity in normal human adults by using power spectral analysis of blood pressure fluctuations in the present study. In addition, we also investigated the effect of fragrance inhalation on sympathetic functions by measurement of plasma catecholamines levels, which provide another assessment of sympathetic activity.

\*Corresponding author. FAX: +81-45-590-6085  
E-mail: shinichiro.haze@to.shiseido.co.jp

## MATERIALS AND METHODS

### *Subjects*

Forty-three healthy females aged 22 to 25 years, who have some experience in using perfumes in their daily life, participated in the present study. The experimental procedures were explained to subjects and a signed document of informed consent was obtained from each subject.

### *Fragrance compounds*

As test samples, we used 6 types of essential oils (natural mixture of fragrance compounds): pepper oil (Robertet, Grasse, France), grapefruit oil (Charabot, Grasse, France), estragon oil (Robertet), fennel oil (Charabot), patchouli oil (Givaudan, Vernier, Switzerland) and rose oil (Toyotama, Tokyo). Pepper oil, estragon oil, fennel oil, patchouli oil and rose oil were obtained from corns of pepper (*Piper nigrum* L.), entire plant of tarragon (*Artemisia dracunculus* L.), seeds of fennel (*Foeniculum vulgare* MILL), leaves of patchouli (*Pogostemon patchouli* PELLETIER. var. SNAVIS HOOK) and flowers of rose (*Rosa damascena* MILL), respectively, by steam distillation. Grapefruit oil was obtained as a by-product of grapefruit (*Citrus paradisi* MACF.) juice production. These essential oils are widely used in fragrance, cosmetic and food industries.

### *Fragrance inhalation*

Each essential oil was separately dissolved at a concentration of 2% (wt/wt) in triethyl citrate (TEC, an odorless solvent). For stimulation, 20  $\mu$ l of the solution was applied to a piece of absorbent cotton (8  $\times$  8 mm), and the cotton piece was fitted under the subject's nose (i.e., on the philtrum) to inhale the fragrance of the essential oil during ordinary breathing. Subjects inhaled the fragrance of essential oils for 3 min in the spectral analysis test or for 7 min in the plasma catecholamine measurement test. In advance of the test, subjects were explained that odorless samples were included, but were not shown what kind of samples were inhaled in the trials.

### *Assessment of sympathetic activity using spectral analysis of blood pressure fluctuations*

Twenty-one healthy female subjects aged 22 to 25 years participated in this experiment. Subjects were instructed to refrain from taking any stimulants such as nicotine and caffeine for 12 h before the test. Subjects were randomized to inhale three of six essential oils. Each subject was tested in three trials. In each trial, the subject inhaled two samples, i.e., essential oil and control (odorless solvent TEC), in a random order. To examine the influence of TEC on sympathetic activity, a cotton piece without any solvent was used as the control.

Subject was seated in a reclining seat and allowed to

rest for 30 min, and then a patient monitor BP-508SD (Colin Medical Instruments, Komaki), which measures arterial blood pressure by using tonometric pulse pressure wave sensor, was fitted for continuous monitoring of heart rate and blood pressure in resting state. After 3 min, a cotton piece soaked with fragrance solution or control was fitted under the subject's nose and the measurement in the fragrance-inhaling state was performed for another 3 min.

Changes in arterial blood pressure were analyzed with an autonomic nerve activity analysis system (Fluclet WT; Dainippon Pharmaceutical, Osaka), which analyzes blood pressure fluctuations by using wavelet transform processing, and the low frequency amplitude (frequency range: 0.04–0.15 Hz) of systolic blood pressure (SBP-LF amplitude) was calculated as an index of sympathetic activity at 5-s intervals (11). The ratio of 2-min average of the SBP-LF amplitudes in fragrance-inhaling state to that of the SBP-LF amplitudes in the resting state was defined as relative sympathetic activity, and this value was used to compare the effect of fragrance with that of control TEC.

### *Assessment of sympathetic activity by measurement of plasma catecholamine levels*

Another twenty-two healthy female subjects aged 22 to 25 years participated in this additional experiment. Subjects were divided into 4 groups to examine the effect of fragrance inhalation on sympathetic activity. Subjects were instructed to refrain from taking any stimulants such as nicotine and caffeine for 12 h before the test. In the test, subjects of each group inhaled pepper oil, grapefruit oil, rose oil or TEC (odorless control) separately; and plasma levels of noradrenaline, adrenaline and dopamine were measured before and after inhalation. Subjects were seated and allowed to rest for 30 min, and then 5 ml of blood was collected from each subject to analyze the level of catecholamines under resting state. A cotton piece was then fitted under the nose of each subject to inhale the fragrance. After 7-min inhalation, 5 ml of blood was withdrawn into an ethylenediaminetetraacetic acid disodium salt (EDTA-2Na)-containing tube and this represented the post-inhalation sample. The blood samples were immediately centrifuged at 4°C, 2,000 rpm for 10 min, and the resulting plasma samples were stored at –80°C until analysis. Plasma levels of noradrenaline, adrenaline and dopamine were measured with the HLC-800 catecholamine analyzer (Tosoh, Tokyo) using the post-column fluorescence high performance liquid chromatography method.

### *GC/MS analysis of essential oils*

Compositions of essential oils used in this study were analyzed by the gas chromatography/mass spectrometry (GC/MS) method. GC/MS analysis was performed with a G1800A GCD system (Hewlett-Packard, Palo Alto, CA,

USA) as follows: column, HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm film thickness, Hewlett-Packard); temperature, 4°C per min (from 60°C to 230°C), 230°C for 50 min; carrier gas, helium, 1 ml per min; ionization voltage, 70 eV. Each peak detected in GC/MS analysis was identified using the WILEY 275 mass spectral database and search program software.

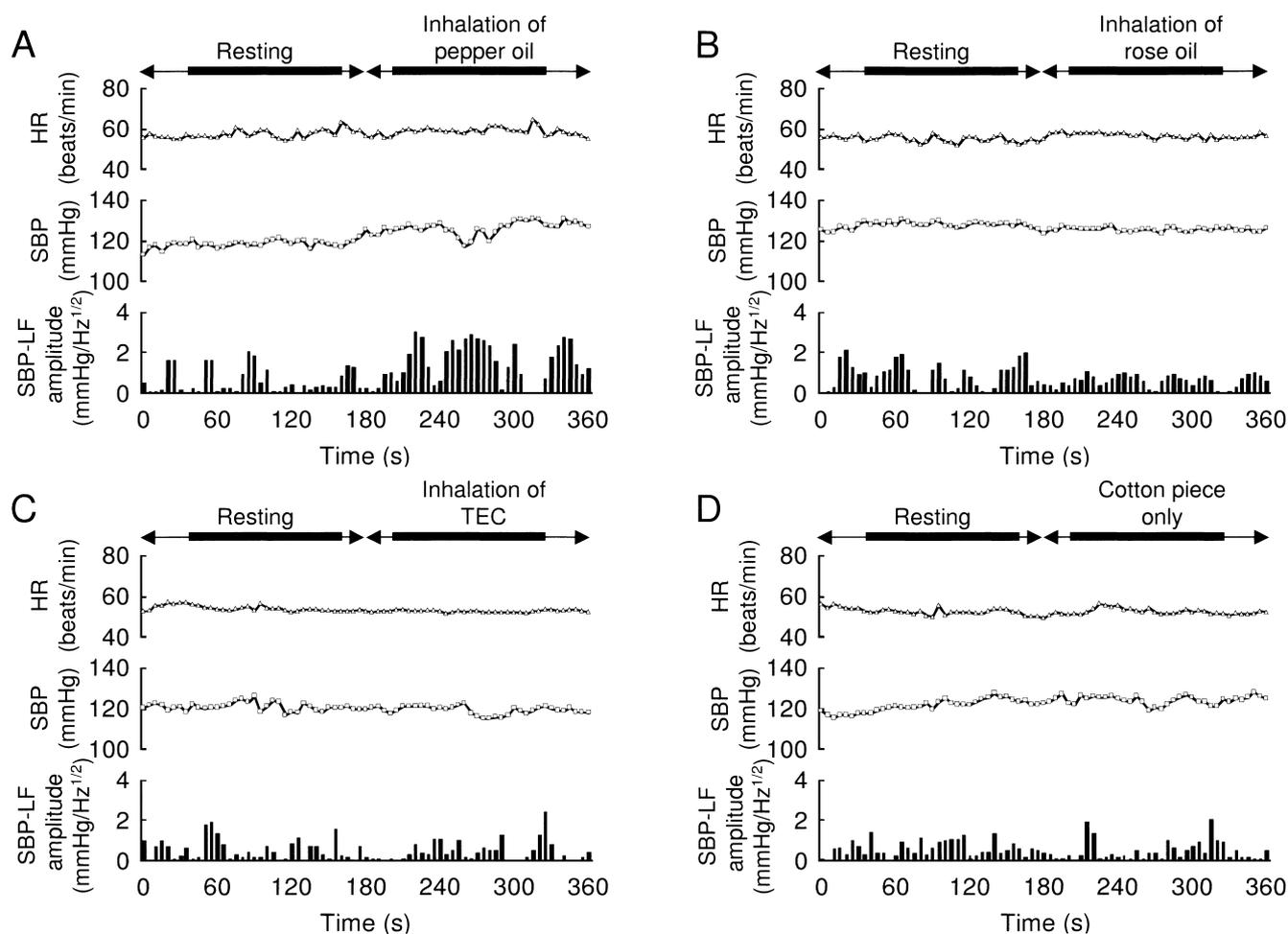
#### Statistical analyses

All data were expressed as the mean ± S.D. Differences between essential oil and TEC in the effects on relative sympathetic activity were examined for statistical significance using Student's *t*-test. Differences between before and after fragrance inhalation in plasma catecholamine levels were examined for statistical significance using the paired *t*-test.

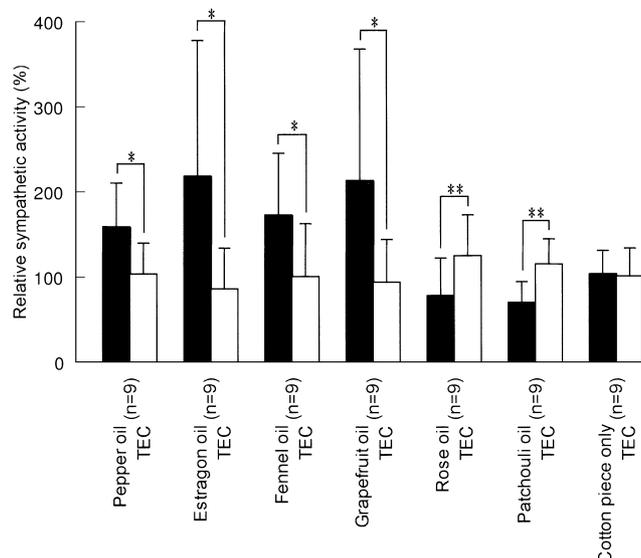
## RESULTS

### Stimulatory and inhibitory effects of fragrance inhalation on sympathetic activity

Figure 1 shows a typical change in SBP-LF amplitude caused by fragrance inhalation of pepper oil or rose oil. Inhalation of fragrance of pepper oil induced a marked increase in SBP-LF amplitude, compared with the level in the preceding resting period (Fig. 1A). In contrast, inhalation of the rose oil induced a considerable decrease in SBP-LF amplitude (Fig. 1B). Odorless TEC or fitting of a cotton piece without any solution produced only a little change in SBP-LF amplitude (Fig. 1: C and D). The ratio of 2-min average of the SBP-LF amplitude in fragrance-inhaling state to the average in preceding resting state was defined as relative sympathetic activity, and this value was used to compare the effect of essential oil with that of control TEC. Fragrance inhalation of pepper oil, estragon oil, fennel oil



**Fig. 1.** Typical changes in sympathetic activity induced by fragrance inhalation. A: Inhalation of pepper oil. B: Inhalation of rose oil. C: Inhalation of odorless solvent triethyl citrate (TEC). D: Control test under conditions where a cotton piece without any solution was fitted. Each bold line shows the period used to calculate the 2-min average of the SBP-LF amplitude.



**Fig. 2.** Stimulant and inhibitory effects of fragrance inhalation on sympathetic activity. The ratio of 2-min average of the SBP-LF amplitudes in fragrance-inhaling state to that of the SBP-LF amplitudes in the resting state was defined as relative sympathetic activity, and this value was used to compare the effect of fragrance with that of control TEC. Open column indicates the relative sympathetic activity in TEC inhalation, and closed column indicates the relative sympathetic activity in essential oil inhalation. Data are mean  $\pm$  S.D. \* $P$ <0.05, \*\* $P$ <0.01.

or grapefruit oil induced 1.7- to 2.5-fold increase in relative sympathetic activity, representing significant differences in relative sympathetic activity between the inhalation of each essential oil and TEC ( $P$ <0.05 each, Fig. 2). In contrast,

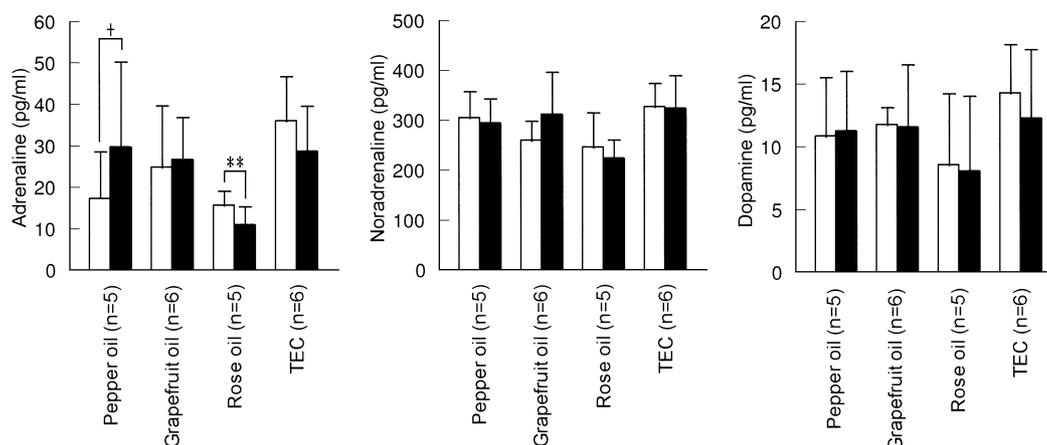
fragrance inhalation of rose oil or patchouli oil resulted in approximately 40% decrease in relative sympathetic activity, and there was a significant difference in relative sympathetic activity between inhalation of rose oil or patchouli oil and the control TEC ( $P$ <0.01, each).

#### *Effect of fragrance inhalation on plasma catecholamine levels*

Changes in catecholamine levels induced by fragrance inhalation of pepper oil, grapefruit oil and rose oil were measured. As shown in Fig. 3, comparison of measured levels before and after inhalation of pepper oil showed a 1.7-fold increase in plasma adrenaline concentration ( $P$ =0.06). Fragrance inhalation of pepper oil showed a tendency to cause an increase in plasma adrenaline level. In contrast, fragrance inhalation of rose oil resulted in a 30% decrease in adrenaline concentration ( $P$ <0.01). Fragrance inhalation of grapefruit oil resulted in 1.1-fold and 1.2-fold increase in adrenaline and noradrenaline levels, respectively, but these changes were not statistically significant.

#### *Composition of essential oils*

The main components of each essential oil detected by GC/MS analysis are shown in Table 1. Terpenes and sesquiterpenes such as pinene, myrcene, limonene and caryophyllene; ethers such as methyl chavicol and anethole; and other components were detected in pepper oil, estragon oil, fennel oil and grapefruit oil, which induced stimulation of sympathetic activity. On the other hand, alcohols such as citronellol, geraniol, nerol and patchouli alcohol, and other components were detected in rose oil and patchouli oil, which induced inhibition of sympathetic activity.



**Fig. 3.** Effects of essential oil inhalation on plasma catecholamine levels. Open column indicates the plasma catecholamine levels before fragrance inhalation (in resting state), and closed column indicates the levels after fragrance inhalation. Data are mean  $\pm$  S.D. + $P$ <0.1, \*\* $P$ <0.01.

**Table 1.** GC/MS analysis of essential oils

Essential oils	Components	Contents (%)
Pepper oil	<i>β</i> -Caryophyllene	21.4
	Limonene	19.5
	<i>α</i> -Pinene	14.6
	<i>β</i> -Pinene	13.3
	<i>δ</i> -3-Carene	12.3
	<i>β</i> -Myrcene	2.2
Estragon oil	Methyl chavicol	59.2
	Linalool	17.7
	Benzyl benzoate	6.2
	Limonene	3.9
	<i>trans-β</i> -Ocimene	2.3
	Eugenol	1.9
Fennel oil	Anethole	70.7
	<i>α</i> -Pinene	7.8
	Fenchone	7.3
	<i>α</i> -Phellandrene	3.2
	Limonene	2.1
	Methyl chavicol	1.4
Grapefruit oil	Limonene	93.3
	Myrcene	1.5
	Sabinene	0.6
	<i>α</i> -Pinene	0.6
Rose oil	Citronellol	27.7
	Geraniol	15.0
	Nonadecane	14.5
	Nerol	7.5
	Linalool	2.4
	Methyl eugenol	1.8
	Phenylethyl alcohol	1.7
Patchouli oil	Patchouli alcohol	42.4
	Seychellene	10.1
	<i>α</i> -Guaiene	8.8
	<i>α</i> -Patchoulene	4.9
	Pogostol	3.0
	<i>β</i> -Caryophyllene	1.3
	<i>α</i> -Gurjunene	1.2

## DISCUSSION

In this study, we investigated the influence of fragrance inhalation on human sympathetic activity using both power spectral analysis of blood pressure fluctuations and changes in plasma catecholamines. Our results showed that fragrance inhalation of pepper oil, estragon oil, fennel oil and grapefruit oil significantly stimulates sympathetic activity measured as SBP-LF amplitude, whereas rose oil and patchouli oil inhibit such activity.

Our results also showed that fragrance inhalation of

pepper oil induced an increase in adrenaline concentration, while inhalation of rose oil decreased adrenaline level. These results were in agreement with the results of power spectral analysis of blood pressure fluctuations. These results suggested that fragrance inhalation affects the adrenal system via the sympathetic nervous system.

Our study demonstrated that noise-adjusted power spectral analysis of blood pressure is a useful method for quantitating the effect of fragrance inhalation on human sympathetic activity. It has been reported that changes in respiration pattern during the test period may affect the results of power spectral analysis of blood pressure fluctuations (12). The autonomic nerve activity analysis system that we adopted for the assessment of sympathetic activity in this study can minimize the effects of changes in respiration pattern by a noise-adjusting system. In this system, outlying observed values derived from irregular breathing can be eliminated from sympathetic nerve activity calculations in real time by digital filters and Smirnov's rejection test (11). Also, we used the relative sympathetic activity, defined as a ratio of 2-min average of the SBP-LF amplitudes in fragrance-inhaling state to that of the SBP-LF amplitudes in resting state, to compare the effect of fragrance with control. We think that these measures brought about sufficient results to examine the effects of fragrance inhalation.

In this study, there were no significant changes in catecholamine levels following fragrance inhalation apart from those of adrenaline. It has been reported that some agents such as caffeine and melatonin have different effects on the plasma levels of noradrenaline and adrenaline, and the changes in noradrenaline and adrenaline levels have not always paralleled each other (13, 14). The fragrance inhalation may exert the predominant effects on the plasma level of adrenaline rather than that of noradrenaline. Further investigation employing time-lapse collection of blood and highly sensitive measurement method such as chemiluminescence high-performance liquid chromatography (15) is necessary to clarify the effects of fragrance inhalation on plasma catecholamine levels.

It is generally known that olfactory sense causes adaptation to odors; i.e., the sensing of odors is strong at first but diminishes over time, in the case that the same odor is continuously smelled. In this study, each subject inhaled one sample odor for a comparatively short period (3 min for power spectral analysis), and the occurrence of adaptation to odors was not observed. It should be examined in the future whether adaptation to odors caused by long-term or consecutive inhalation of fragrance might affect the effects of essential oils on sympathetic activity.

Essential oils with a stimulatory effect on sympathetic activity, e.g., pepper oil, estragon oil, fennel oil and grapefruit oil, contain some common components such as

limonene, pinene or methyl chavicol, and it is possible that these components mediate the stimulatory effect on sympathetic activity. On the other hand, no common components were found in the inhibitory essential oils such as rose oil and patchouli oil, but the characteristic components such as patchouli alcohol, citronellol or geraniol might themselves be involved in mediating the inhibitory effect. It is therefore important to determine the active components that contribute to the stimulatory and inhibitory effects on sympathetic activity in future studies.

Mechanisms of the effects of these fragrances on sympathetic activity are not yet clear. One possibility that explains the effects of fragrance inhalation on sympathetic activity is mental and emotional effects caused by olfactory stimulation. It has been reported that effects of fragrance inhalation on autonomic nervous system parameters, such as skin temperature and blood pressure, are partially affected by mental and emotional conditions (6). These psychological responses to an odorant might also be influenced by acquired experience and memory. It is conceivable that pleasant fragrances might induce relaxation and decrease sympathetic activity, and unpleasant ones may increase it. Six essential oils tested in this study are commonly used in cosmetics and foods, and all these fragrances give familiar and pleasant impressions to the subjects, according to our questionnaire surveys. So we think that it is unreasonable to attribute the effects of fragrance inhalation on sympathetic activity to mere mental and emotional effects or the effects of acquired experience and memory. Another possibility that explains the effects of fragrance inhalation on sympathetic activity is pharmacological action of essential oil components.

It is assumed that blood pressure fluctuations are generally attributed to the delay in sympathetic vasomotor regulation mediated by the baroreceptor reflex (16, 17). In our studies, fragrance inhalation of grapefruit oil at concentrations of 1% to 10% induced a concentration-dependent increase in SBP-LF amplitude (unpublished data). This observation suggests a possibility that some physiological interactions between essential oil components and biomolecules such as receptors should be engaged in mediating the effects of fragrance inhalation. As an example of the interaction between fragrance materials and receptors, recent studies reported that essential oils bind to the potentiation-sites in ionotropic  $\gamma$ -aminobutyric acid (GABA<sub>A</sub>) receptors and increase the affinity of GABA to the receptors, and they suggested the possibility that fragrance inhalation modulates the neural transmission in brain through ionotropic GABA<sub>A</sub> receptors (18). To prove this possibility, further studies such as assessment of the amounts of fragrance components in blood and brain should be conducted in the future.

Conceivably, both physiological and psychological

mechanisms are involved in the effects of fragrance inhalation on sympathetic activity. In conclusion, we have demonstrated in the present study that fragrance inhalation of essential oil can stimulate or depress sympathetic activity in normal adults. Our findings suggest a possibility that fragrance inhalation may be utilized as a mild regulator of dysfunctions of the sympathetic nervous system.

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