Introduction

Some compounds extracted from several plants have recently shown effects on feeding behaviour, and studies on these compounds have since been conducted to examine whether these compounds can contribute to the prevention of lifestyle diseases [1–3]. For example, geranium essential oil administered via inhalation, but not via oral administration, was found to reduce food intake in rats. These studies investigated the expression levels in the hypothalamus of POMC and NPY, which are involved in the mechanisms of feeding behaviour. Some of the studies revealed that certain compounds and essential oils significantly increased mRNA expression of POMC, which has been linked to a decrease in food intake [1,3], while other studies demonstrated that some compounds and essential oils lowered mRNA expression of NPY, which is believed to increase the food intake [2,3].

Apart from efforts to reduce food intake in healthy people, there is a need to prevent diseases caused mainly by malnutrition in many elderly and infirm people. A non-invasive method of improving appetite would be beneficial for improving their quality of life and primary care [4]. In Japan, over-the-counter medicines administered orally to treat loss of appetite (anorexia) often contain herbal aromatic stomachics such as the bark of Cinnamomum cassia Blume (Lauraceae) and the fruit of Foeniculum vulgare Miller (Umbelliferae). These fragrant herbal medicines contain many essential oils, and their fragrances are suggested to contribute to their appetite-enhancing effects [5].

Abstract

Fragrance in the air and odours of foods and drinks are reported to affect feeding behaviours of humans and other animals. Many previous studies focusing on the relationship between fragrance and appetite have described a reduction of food intake by fragrance administration to help prevent lifestyle diseases. Aromatic herbal medicines, such as cinnamon bark and fennel fruit, are considered to have appetite-enhancing effects and they are often blended in stomachics for relief of asitia and gastric distress in Japan. These fragrant herbal medicines contain many essential oils and their fragrances are hypothesised to be active substances. In this study, food intake and the expression of neuropeptide Y and proopiomelanocortin in the hypothalamus after inhalation of fragrant compounds or essential oils were investigated in mice. Food intake was increased 1.2-fold and the neuropeptide Y mRNA expression in the hypothalamus was increased significantly in mice that inhaled trans-cinnamaldehyde, benzylacetone or 1-phenyl-2-butanone, compared with the control group. These compounds might be effective for treating loss of appetite (anorexia) or eating disorders in elderly and infirm people via a non-invasive route of administration, namely, inhalation.

Abbreviations

NPY: neuropeptide Y
POMC: proopiomelanocortin

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The expression levels of NPY and POMC mRNAs in unfasted mice compared with ghrelin, respectively. The amount of food intake was significantly increased in fasted mice administered trans-cinnamaldehyde, benzylacetone or 1-phenyl-2-butaneone compared with animals in the control group. The amount of food consumed by the mice administered trans-cinnamaldehyde and benzylacetone was approximately 1.2-fold higher than that of the control group (Fig. 2). Ghrelin, the positive control compound, significantly increased food intake in mice compared with the vehicle; however, food intake was increased in mice administered trans-cinnamaldehyde, benzylacetone or 1-phenyl-2-butaneone by 1.07-, 1.09- and 1.06-fold compared with ghrelin, respectively.

The expression levels of NPY and POMC mRNAs in unfasted mice hypothalamus after inhalation of the fragrances are shown in Fig. 3. The mRNA expression of NPY was significantly elevated in the same groups of mice that showed a higher food intake compared with controls (Fig. 2), whereas the expression of POMC appeared to be unrelated to the change in food intake. However, the amount of food intake was about 1.2-fold higher in unfasted mice administered trans-cinnamaldehyde or benzylacetone compared with the unfasted mice in the control group (Fig. 3), although due to dispersion of data, this relationship was not significant. Here, the ratio of increase was similar in fasted and unfasted mice.

Discussion

Among the fragrant compounds examined in this study, those possessing a phenylpropanoid structure showed positive effects on appetite enhancement in mice, whereas such effects were not observed in compounds that either did not have carbonyl groups or did have these groups but in the adjacent position to the phenyl group. In other studies, similar structure-activity relationships for carbonyl groups and the length of aliphatic chains have been reported [8, 9]. Furthermore, (R)-linalool and geranium essential oil, which are rich in monoterpenes compounds such as geraniol and citronellol (Table S1, Supporting Information), did not show effects on appetite enhancement. The putative mechanisms of any effects induced by fragrant compounds administered via inhalation include two pathways: (i) the stimulation of receptors in the central nervous system by compounds being absorbed and transferred to blood and (ii) the direct stimulation of olfactory receptors [1, 10–12]. In some studies it was demonstrated that the effects were abrogated by olfactory impairment [1, 11]. To clarify the mechanisms of action of the appetite-enhancing effects, benzylacetone was dissolved and diluted in corn oil (Wako Pure Chemical Industries, Ltd.) and administered intraperitoneally (1.0, 0.1, 0.01 µg/kg) to mice. However, significant effects were not observed in any group (Fig. 4). This finding suggests that the appetite-enhancing effects might not be caused by the compound transferred to the blood stream, but instead by direct olfactory stimulation.

The results shown in Figs. 2 and 3 indicate that the enhancement of food intake seemed to be positively correlated with the changes in NPY mRNA expression. Comparing Figs. 2 and 3 indicates that the appetite-enhancing effects were similar whether the mice were fasted or not; therefore, comparing Figs. 2 and 3 was considered to be appropriate. This could imply that NPY mRNA expression was significantly increased by the administration of trans-cinnamaldehyde, benzylacetone or 1-phenyl-2-butaneone via inhalation, and indeed hypothalamic NPY is known as a peptide that stimulates food intake [13].

![Fig. 1](https://example.com/fig1.png) Compounds tested in this study. 1. trans-Cinnamaldehyde, 2. benzylacetone, 3. 1-phenyl-2-butaneone, 4. butylbenzene, 5. 6-methyl-5-hepten-2-one, 6. benzaldehyde, 7. (R)-linalool.

![Fig. 2](https://example.com/fig2.png) Appetite enhancement in fasted mice by inhalation of fragrant compounds. a 4.5 × 10⁻³ mg/cage, b 4.5 × 10⁻⁴ mg/cage, c 4.5 × 10⁻⁵ mg/cage. Vehicle: saline injected i. p.; positive control: ghrelin; control: triethyl citrate inhalation. Data are expressed as mean ± SEM (n = 8 mice). Statistical analysis was performed using subsequent t-test with Bonferroni correction for multiple comparisons and one-way analysis of variance (ANOVA) followed by Dunnett’s test. *P < 0.05 and **P < 0.01 vs. control group; #P < 0.01 vs. vehicle group.

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was previously reported that the expression of NPY decreased as that of POMC increased, and food intake decreased [3]. However, the present results do not concur; the expression of NPY significantly increased when food intake also increased, and the expression of POMC decreased at the same time, albeit not significantly, when food intake increased. The expression levels of NPY and POMC did not significantly contradict to the results of previous studies when fragrant compounds were administered by inhalation, although the expression level of POMC tended to decrease. The putative mechanism of appetite-enhancing effects and the relation between food intake and mRNA expression in the hypothalamus suggest that appetite-enhancing could be caused by the stimulation of the olfactory receptor followed by an increase in NPY and stimulation of the NPY receptor.

Compounds such as Δ9-tetrahydrocannabinol, diazepam [14], anandamide [15], morphine [16], and ghrelin [17] were previously reported to have appetite-enhancing effects. Of note, Δ9-tetrahydrocannabinol, diazepam and morphine have addictive properties; therefore, regulation of these compounds is very strict. Thus, a repetitive or continuous use of these compounds for anorexia would be problematic [18–21]. Ghrelin could be administered via injection, although it has stability problems during long-term storage and is unstable even if it is kept at low temperatures with stabilizing agents and protease inhibitors [22]. Compared with these compounds, trans-cinnamaldehyde, benzylacetone and 1-phenyl-2-butanone are unique in that they could be administered non-invasively via inhalation, and are expected to improve anorexia or eating disturbances of elderly and infirm individuals.

Recently, it was reported that a Kampo medicine, rikkunshito, increased plasma ghrelin levels in rats administered cisplatin and promoted recovery from anorexia [23]. Another experiment showed that ghrelin increased the expression of NPY mRNA in the hypothalamus of rats [24]. Because both benzylacetone and ghrelin increase the expression of NPY mRNA in the hypothalamus, benzylacetone could be used for conditions similar to those treated with rikkunshito.

In conclusion, trans-cinnamaldehyde, benzylacetone and 1-phenyl-2-butanone displayed appetite-enhancing effects via inhalation at doses of 4.5 × 10^{-4} mg/cage. The common features of these active compounds are the presence of both carbonyl and phenyl groups in non-adjacent positions. mRNA expression of NPY in the hypothalamus of mice was increased when these compounds were administered via inhalation. However, appetite-enhancing effects were not observed when the mice were administered benzylacetone intraperitoneally. These results indicate that the stimulation of olfactory receptors on nasal mucosa by inhaled compounds may increase the mRNA expression of NPY in the hypothalamus, leading to an enhancement of food intake. Comparing the results of this study with those reported by Choi et al. [1], it is interesting that both, benzylacetone and geranium essential oil, showed their effects by stimulating olfactory receptors, even though they produced opposite effects.

To our knowledge, there have been only three reported studies of the effects of fragrant compounds on food intake [1, 3, 25], all showing a reduction in food intake. The doses in the reported studies were significantly higher than in our study. For example, the amount of geranium oil used in Choi et al.’s study [1] was estimated to be 20 µL/cage (6.4 × 10^{-4} µL/cm³), which is much higher than the dose of 4.5 × 10^{-3} mg/cage (7.6 × 10^{-8} mg/cm³) used here. The high dose of the
oils could increase the expression level of POMC mRNA [1,3], which may increase the levels of α-melanocyte stimulating hormone formed by metabolism of POMC. α-Melanocyte stimulating hormone reduces the appetite-enhancing effects of NPY [13]; therefore, both the decrease in food intake caused by higher doses and the appetite-enhancing effect of lower doses can be explained.

The three compounds investigated in this study have characteristics of food intake enhancement: they have a pleasant smell, are chemically stable, easy to synthesise and therefore might be good candidates for functional fragrances that might attract people to foods with these flavours. Herbal medicines or spices containing phenylpropanoid compounds, including cinnamon, were used to treat anorexia [26], and it was suggested that the component compounds of these spice essential oils [5], such as trans-anethole, stimulated olfactory receptors different from the ones stimulated by trans-cinnamaldehyde [27]. This suggests that some compounds that have structural features different from trans-cinnamaldehyde or benzaldehyde might enhance the appetite via inhalation. Accordingly, the appetite-enhancing effects of other phenylpropanoid compounds contained in spices and herbal medicines should be clarified in humans in addition to animals.

Materials and Methods

Materials

Essential oils and compounds used in this study (Fig. 1) were obtained as follows. trans-Cinnamaldehyde and benzaldehyde were purchased from Nacalai Tesque, benzylcinnamate, 1-phenyl-2-butanone, butylbenzene and 6-methyl-5-hepten-2-one were purchased from Tokyo Chemical Industry. Geranium essential oil was purchased from Tisserand and (R)-linalool was obtained from Sigma-Aldrich. All fragrant compounds used in this study were of the highest grade available. Triethyl citrate was purchased from Merck KGA, and 2-mercaptoethanol and ethanol were from Nacalai Tesque. Ghrelin (rat) was purchased from Peptide Institute Inc.

Animals

The animal studies were designed according to the recommendations of the Animal Research Committee of Kyoto University, Kyoto, Japan (authorisation numbers: 2014–17). Four-week-old male ddY mice (approximately 16 g at the time of purchase) were obtained from Japan SLC. The mice were housed in colony cages (six mice per cage) at an ambient temperature of 25 ± 2°C under a 12 h light–dark cycle. The mice used for the measurement of gene expression in the hypothalamus (25 g at the time of experiments) were fed standard pellet chow and water ad libitum. The mice used in the feeding tests (19 g at the time of the experiments) were fasted for up to but not more than 24 h before starting the tests, but water was available ad libitum. All studies were conducted from 08:00 to 17:00.

Feeding test

Feeding tests were performed according to the previous studies of Choi et al. (2011) and Miyoshi et al. (2013) [1,8]. Fragrant compounds and essential oils were dissolved in and diluted with triethyl citrate (400 µL) and dropped onto four filter paper discs attached to the four corners of the glass cage (30 cm × 60 cm × 34 cm; 61.2 L). The cage was filled with vapourised solution by diffusion for 60 min. The fasted mice inhaled fragrances in the cage for 1 h before the end of the fast. At the end of fast, the mice were taken from the glass cage and placed in plastic cages and then given approximately 10 g of weighed standard pellet chow. After 4 h of feeding, they were removed from the plastic cages and the remaining pellets were weighed to calculate the food intake. The control group inhaled air after placement of triethyl citrate in the cage. Ghrelin (rat), a hormone that has an appetite-enhancing effect, was dissolved in saline (Otsuka Pharmaceutical) and injected intraperitoneally (158 nmol/kg) [28] as a positive control 1 h before starting the feeding test and saline was injected as a vehicle.

Evaluation of gene expression in mice hypothalamus

The evaluation method for gene expression used in this study was modified from that described previously [1,10,29]. The mice that were not fasted inhaled the fragrance in their cage for 60 min, then their brains were removed after cervical dislocation and decapitation. Hypothalamus was isolated from the brain and was ground up using liquid nitrogen. Total RNA was extracted from the hypothalamus using an RNeasy fibrous tissue mini kit (Qiagen). cDNA was synthesised using Revatra Ace (Toyobo) and oligo (dT)15 primer (Takara Bio Inc.) with extracted total RNA as a template, and the resultant cDNA was refined by NucleoSpin oligo (dT)15 primer (Takara Bio Inc.) with extracted total RNA as a template, and the resultant cDNA was refined by NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL). Gene expression in the hypothalamus was measured using THUNDERBIRD SYBR qPCR Mix (TOYOBO) on StepOnePlus™ (Applied Biosystems). Quantitative real-time PCR was performed with a temperature programme starting at 95.0°C for 20 s, followed by 40 cycles of 95.0°C for 3 s, 45.0°C for 30 s and 72.0°C for 30 s. The primers used in this study are shown in Table 1. Calibration curves for each couple of primers were plotted using the results of real-time PCR. The calibration curves of β-actin, POMC and NPY and their correlation coefficients are shown in Table 1. The linearity of the calibration curves was evaluated with the correlation coefficients, and the appropriate values were set to > 0.98 [8]. The effi-
ciency of the target amplification was calculated based on the slopes of the calibration curves, and the efficiency was confirmed to be 0.8–1.2 [30]. Quantitative evaluation of gene expression was analysed using the 2^(-ΔΔCt) method [31].

**Statistical analysis**

Results are presented as the mean ± standard error of the mean (S.E.M.). Statistical analyses were performed by Dunnett’s test and subsequent t-test with Bonferroni comparison for multiple comparisons [1, 8] using GraphPad Instat (GraphPad Software) and R (R Development Core Team, R Foundation for Statistical Computing). A probability level of p < 0.05 was considered statistically significant [8].

**Supporting information**

Analysis results for geranium essential oil and a description of the GC-GC/MS analysis methods are available as Supporting Information.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**

3. Ishihara M, Tsuneya T. Analysis of relative gene expression data using the 2^(-ΔΔCt) method [31].
22. Delhanty PD, Huisman M, Julien M, Mouchain K, Brune P, Themmen APN, Abribat T, van der Lely AJ. The acylated (AG) to unacylated (UAG) ghrelin ratio in esterase inhibitor-treated blood is higher than previously described. Clin Endocrinol (Oxf) 2014; 82: 142–146
27. Furudono Y, Sone Y, Takizawa K, Hirono J, Sato T. Relationship between peripheral receptor code and perceived odor quality. Chem Senses 2009; 34: 151–158