Effect of the Scent from Grapefruit Oil on Salivary Secretion in Rats

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Abstract
It has been noted that the fragrance of grapefruits has a refreshing and exciting effect, which suggests the activation of sympathetic nerves. In salivary glands, the activation of sympathetic or parasympathetic nerves induces the secretion of proteins (such as the digestive enzyme \( \alpha \)-amylase (amylase) and the glycoprotein mucin) or water and ions, respectively. Here, we investigated the effects of the scent from grapefruit oil on body weight and food intake, and its acute effects on salivary fluid and amylase secretion into the oral cavity of rats. When rats were exposed to the scent from grapefruit oil for 5 weeks, their body weight and food intake decreased. When rats were treated with the scent for 20 min, salivary amylase secretion was enhanced without changes in fluid secretion. These findings suggest that the scent from grapefruit oil activates sympathetic nerves, but not parasympathetic nerves, resulting in an anti-obesity effect.

Keywords:
essential oil, grapefruit, amylase secretion, salivary glands

Introduction
Plant essential oils have been used as major fragrances for olfactory stimulation. It has been reported that fragrances of the citron group activate brain function and mental activity (1), while the peppermint fragrance limits the increase in daytime sleepiness in humans (2). The cedrol fragrance decreases heart rate and blood pressure in humans (3), while jasmine and lavender fragrances decrease heart rate (4). These reports suggest that plant essential oils regulate biological reactions via autonomic nervous functions. In fact, the lavender fragrance has been shown to increase \( \beta \)-adrenergic responses, while the rosemary fragrance decreases \( \alpha \)– and \( \beta \)-adrenergic responses in humans (1). Chamomile and lavender fragrances decrease \( \alpha \)-adrenergic responses in humans (5). Grapefruit and lemon fragrances have been reported to increase sympathetic nervous responses in humans (6) and in rats (7).

Salivary glands are exocrine glands, and three anatomically distinct pairs of major salivary glands (the parotid, submandibular, and sublingual glands) produce and secrete saliva into the oral cavity. The cells of salivary glands are involved in two different processes, the production of the primary secretory fluid, and the secretion of proteins, such as the digestive enzyme \( \alpha \)-amylase (amylase) and the glycoprotein mucin (8). Both of these cellular processes are controlled by the autonomic nervous system. In salivary glands, stimulation of the parasympathetic nerve leads to the secretion of salivary fluid, whereas stimulation of the sympathetic nerve leads to exocytotic secretion of salivary proteins (8). In the present study, we investigated the effects of the scent from grapefruit oil, a plant essential oil, on metabolic regulation and its acute effects on salivary fluid and amylase secretion in rats.

Methods

Animals
Male Sprague–Dawley rats (12 weeks of age) were used for these studies (control group, \( n=7 \); experimental group, \( n=7 \)). All animals were housed in a room maintained at 24±1°C and illuminated for 12 h
(07:00 – 19:00). Food (type MF; Oriental Yeast Co., Tokyo, Japan) and water were freely available. Procedures for animal care and handling were approved by the Institutional Animal Care and Use Committee of the Nihon University School of Dentistry at Matsudo. Experiments were conducted after allowing the animals to adapt to their housing conditions for 1 week.

Effects of the scent from grapefruit oil on body weight and food intake

The scent from grapefruit oil (Gaia, France, 10 µl) placed on the side of each animal cage (25 × 35 cm, 15 cm high) at 16:00 every Monday for 5 weeks. Body weight and food intake were measured every week. To avoid leakage of the scent from grapefruit oil, each cage was covered with vinyl. Rats exposed similarly to water were used as controls.

Effects of the scent from grapefruit oil on salivary fluid secretion

Rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and then were exposed to the scent from grapefruit oil according to the method of Niijima and Nagai (7). Briefly, to examine the effect of the scent from grapefruit oil on salivary secretion, a filter paper (φ 40 mm) soaked either in water or in grapefruit essential oil was placed in the bottom of a beaker (diameter, 5 cm; depth, 6 cm). The nose of the anesthetized rat was placed inside the beaker for 20 min, and saliva was absorbed onto paper plugs inserted into the oral cavity. The saliva–saturated plugs were weighed, and corrected for the original weight of the plug. The volume of secreted saliva was obtained as the increase in weight of the paper plug. Rats treated similarly with water were used as controls.

Amylase assay

Saliva–saturated plugs were immersed in phosphate buffered saline (pH 6.9) for 15 min. Amylase activity in the medium was then measured according to the method of Bernfeld (9), modified for incubation at 30°C for 5 min. One unit of amylase is defined as the quantity of enzyme that liberates 1 mg maltose/min at 30°C, according to the method of Fujita–Yoshigaki et al. (10).

Statistical analysis

Statistical differences were determined using Student’s t-test. P values below 0.05 or 0.01 were regarded as statistically significant.

Results

Effect of the scent from grapefruit oil on body weight and food intake

We examined the effect of the scent from grapefruit oil on body weight and food intake in male rats (Fig. 1). Rats were exposed to grapefruit oil, which was placed on the animal cage every week for 5 weeks. The body weight of rats exposed to the scent from grapefruit oil was similar to that of rats exposed to water for the first week; however, by the beginning of the second week, the body weight of the rats exposed to grapefruit oil was significantly lower than that of the control rats (Fig. 1A). Similarly, the food intake of rats exposed to the scent from grapefruit oil was significantly reduced from the second week until the end of the study compared to that of the control rats (Fig. 1B). These results suggest that the scent from grapefruit oil may have an anti-obesity effect in rats.

Effect of the scent from grapefruit oil on salivary fluid secretion

We studied the acute effect of the scent from grapefruit oil on salivary fluid secretion in pentobarbital–anesthetized rats (Fig. 2A). Rats were exposed to filter paper soaked with the essential oil of grapefruit for 20 min. There was no difference in the amount of saliva secreted between rats exposed to grapefruit oil or to water (control), suggesting that the scent from grapefruit oil does not induce salivary fluid secretion in rats.

Effect of the scent from grapefruit oil on salivary amylase secretion

We subsequently determined the acute effect of the
scent from grapefruit oil on salivary amylase secretion by pentobarbital-anesthetized rats (Fig. 2B). Rat saliva amylase, absorbed onto paper plugs, was extracted into the medium and measured. Amylase activities (mU/mg) in rats exposed to the scent from grapefruit oil or to water (control) were 50.50±3.54 and 15.31±3.11, respectively. Thus, amylase activity
was significantly elevated in rats exposed to the scent from grapefruit oil compared to the control rats, which suggests that the scent from grapefruit oil induces salivary amylase secretion in rats.

Discussion

In the present study, we demonstrate that the scent from grapefruit oil induces a decrease in body weight and food intake in rats. It has been reported that the scent of grapefruit oil stimulates sympathetic nerves in brown adipose tissue (11) and elevates body temperature (12). In brown adipose tissue, stimulation of the sympathetic nerves activates uncoupling protein (UCP), which increases body temperature and energy consumption (13). Fatty acids are provided via the increase in body temperature and energy consumption (13). Therefore, it is likely that the anti-obesity effect induced by the scent from grapefruit oil is caused by an enhancement of lipolysis, as previously demonstrated (7). On the other hand, the scent from grapefruit oil has been reported to reduce the activation of gastric parasympathetic nerves (12). Since activation of gastric parasympathetic nerves induces gastric peristalsis, it is conceivable that the scent inhibits gastric peristalsis and consequently decreases appetite. These observations strongly suggest that the scent from grapefruit oil activates the autonomic nervous system, which contributes to its anti-obesity effect.

We also demonstrate that the scent from grapefruit oil acutely induces amylase secretion, but not fluid secretion into the oral cavity (Fig. 2). In major salivary glands, activation of sympathetic nerves provokes secretion of salivary proteins, whereas activation of parasympathetic nerves dominantly induces fluid secretion (8). In rat parotid acinar cells, stimulation of the sympathetic nerve induces amylase secretion via activation of β-adrenergic receptors (14). Therefore, our observations suggest that activation of sympathetic nerves in parotid glands by the scent results in amylase secretion from the acinar cells. In rats acutely exposed to the scent from grapefruit oil, an increase in respiratory rate, one of the functions of the sympathetic nervous system, also occurred (data not shown), which supports the idea that the scent acutely activates sympathetic nerves. However, since the scent failed to increase salivary fluid secretion, the acute effect of the scent on parasympathetic nerves seems to be weak. Therefore, such an acute activation of sympathetic nerves appears to be effective in enhancing metabolic regulation and include anti-obesity effects.

Recently, therapy with plant essential oils has been gradually innovated at the bedside. However, the effects of plant essential oils are sometimes poorly reproducible because the quality of oil ingredients is naturally variable. Salivary amylase has been proposed as a sensitive biomarker for sympathetic nervous function for clinical tests (15). Our findings strongly suggest that determining amylase activity in saliva is useful to check the effects of oils on autonomic nervous functions.

In conclusion, the scent from grapefruit oil stimulates sympathetic nerves within normal reactions, which then moderately enhances metabolism, resulting in an anti-obesity effect. By checking the amylase activity in the saliva, enhancements of metabolism due to the scent from grapefruit oil can be detected.

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References