Effect of Olfactory Stimulation with Flavor of Grapefruit Oil and Lemon Oil on the Activity of Sympathetic Branch in the White Adipose Tissue of the Epididymis

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It has been mentioned in the field of aromatherapy that the fragrance of grapefruit has a refreshing and exciting effect, which suggests an activation of sympathetic nerve activity. It also can be assumed that the activation of sympathetic nerve innervating the white adipose tissue (WAT) facilitates lipolysis, then results in a suppression of body weight gain. This study addressed the effect of olfactory stimulation with the scent of grapefruit oil and lemon oil on the efferent nerve activity in the sympathetic branch of the WAT of the epididymis in the anesthetized rat. The results of the experiments demonstrated that the flavor of the citron group increased sympathetic nerve activity to WAT in anaesthetized rat, which suggests an increase in lipolysis and a suppression in body weight gain. Exp Biol Med 228:1190–1192, 2003

Key words: flavor; sympathetic nerve; adipose tissue; aromatherapy

In an article of aromatherapy it is mentioned that the flavor of the citron group, including grapefruit, activates brain function and mental activity (1), which may evoke the activation of the sympathetic outflow. It is supposed that the sensory signals originated by the aromatic flavor in the olfactory mucous membrane propagate in the olfactory nerve to the limbic system as well as autonomic center in the hypothalamus through the olfactory bulb. It will be reasonable to speculate that the activity of the sympathetic nerve can be modulated by these sensory signals.

It is recognized that noradrenaline liberated at the endings of the postganglionic sympathetic nerves supplying the fat depots can cause tryglyceride hydrolysis. This suggests that there is a direct neural control of fat metabolism *via* the sympathetic nervous system, which has been reviewed by Shimazu (2). In this respect, it can be assumed that the activation of sympathetic nerve innervating the white adipose tissue (WAT) may facilitate lipolysis, then result in a suppression of body weight gain.

This study addressed the effect of olfactory stimulation with the scent of grapefruit and lemon on the efferent nerve activity in the sympathetic branch of the WAT of the epididymis in the anesthetized rat.

Materials and Methods

Male Wistar rats, weighing about 300 g, were used. They were kept in a room maintained at 24°C with illumination for 12 hr per day (0700 hr–1900 hr). Food and water were freely available until the day of the experiment. On the experimental day food, except for water, was removed 6 hr before the experiment. The animals were anesthetized by ip injection of 1 g/kg urethane. The efferent nerve activity was recorded from a nerve filament dissected from the central cut end of the sympathetic branch innervating the WAT of the epididymis (3–5). The recording electrodes were immersed in a mixture of liquid paraffin and petroleum jelly to prevent dehydration. The nerve activity was amplified in a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. All nerve activity was analyzed after conversion of raw data to standard pulses by a window discriminator, which separated discharges from background noise. The discharge rate was displayed on a pen recorder by means of a rate meter with reset time of 5 sec. For the olfactory stimulation, grapefruit oil (*Citrus paradisii*, Pranarom, France) or lemon oil (*Citrus lemon*, Pranarom, France) was diluted with distilled water for 10,000~100 times. A filter paper was soaked in the solution and placed on the bottom of a beaker (diameter, 5 cm; depth, 6 cm). For the olfactory stimulation, the nose of the rat was placed inside of the beaker for 10 min (Fig. 1). The effects of olfactory stimulation were investigated by comparing the mean number of impulses per 5 sec over 50 sec (i.e., mean value of 10 successive measured samples) before

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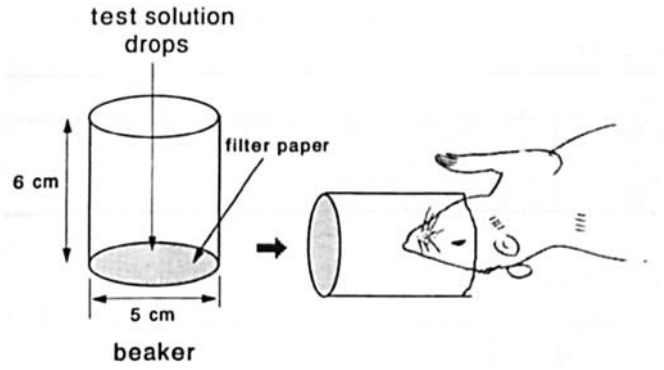

Figure 1. Olfactory stimulation in anesthetized rat (see text).

and after the stimulation. Data are expressed as the mean ± SEM. Statistical significance was determined by analysis of variance ($P < 0.05$).

Results

Figure 2 presents typical examples of the effect of olfactory stimulation with flavor of grapefruit oil and lemon oil for 10 min on the efferent nerve activity of the sympathetic branch innervating the WAT of epididymis. As shown in the top trace, an olfactory stimulation with grapefruit oil (diluted 1000 times) for 10 min evoked a slight increase in nerve activity; however, a thinner solution (1/10,000 in concentration) presented no clear response. In the second trace, it was demonstrated that a flavor stimulation with grapefruit oil with 1/100 in concentration caused a gradual and remarkable increase in nerve activity that lasted longer than 90 min. The olfactory stimulation with lemon oil diluted for 100 times also demonstrated a clear increase

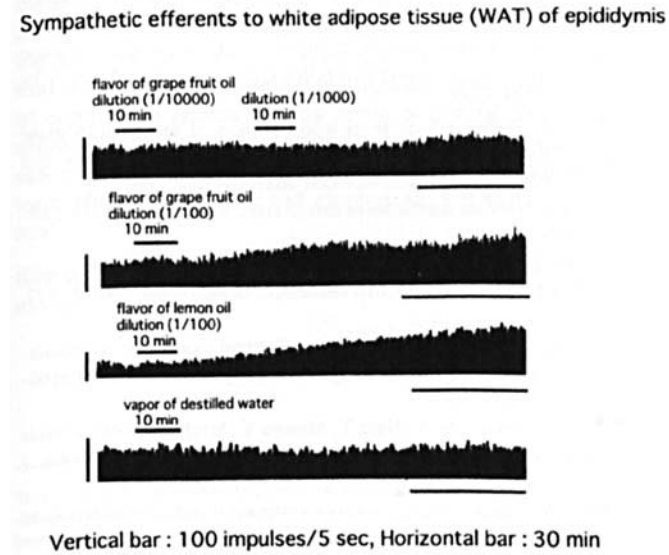

Figure 2. Effect of olfactory stimulation with grapefruit oil and lemon oil on the efferent activity of the sympathetic nerve to the WAT of the epididymis. Upper trace, Olfactory stimulation with grapefruit oil diluted 10,000 times and 1000 times. Second trace, Stimulation with grapefruit oil diluted 100 times. Third trace, Stimulation with lemon oil diluted 100 times. Bottom trace, Olfactory stimulation with water vapor.

in nerve activity (third trace). On the contrary, an olfactory stimulation with the vapor of distilled water for 10 min was without effect. Table I and Figure 3 show the mean discharge rates of the above-mentioned sympathetic nerve activity to the WAT of epididymis before and after olfactory stimulation with grapefruit and lemon flavor ($n = 5$, $P < 0.05$; asterisk indicates significant increase). The mean discharge rates of just before and 30, 60, and 90 min after stimulation with grapefruit oil and lemon oil (dilution, 100 times) were 68.1 ± 4.5, 81.0 ± 7.7*, 82.9 ± 6.4*, and 104.1 ± 8.4* impulses/5 sec, and 67.8 ± 4.6, 80.3 ± 3.6, 92.1 ± 6.2*, and 90.2 ± 9.6* impulses/5 sec, respectively. The mean discharge rates just before and 30, 60, and 90 min after water vapor stimulation were 60.6 ± 2.5, 57.9 ± 1.1, 58.0 ± 2.0, and 59.4 ± 1.6 impulses/5 sec, respectively (Table I). The results indicate that olfactory stimulation with grapefruit oil and lemon oil caused a significant increase in sympathetic nerve activity; however, no significant change in discharge rate was recognized by water vapor stimulation. These observations clearly demonstrate that olfactory stimulation with flavor of the citron group (grapefruit and lemon) results in reflex activation in sympathetic outflow to the WAT of epididymis, which suggests an acceleration of lipolysis in WAT and a suppression in body weight gain.

Discussion

The results of experiments indicate that the sensory signals originating in the olfactory mucous membrane by the olfactory stimulation with the flavor of the citron group arrive at the autonomic center in the hypothalamus through olfactory nerve, then activate sympathetic outflow to the WAT of the epididymis. As described in a review (6), the central nervous system, especially the hypothalamic region, is involved in regulation of fat mobilization from the adipose tissues. It was reported that the mobilization of free fatty acids (FFA) following various stresses in animals is reduced with hypothalamic lesions (7). The electrical stimulation of the hypothalamus increases plasma glycerol and FFA, suggesting the contribution of hypothalamus to the activation of the sympathetic nerves innervating adipose tissues (8, 9). Further, it has been reported by Correll (10) and Shimazu (2) that direct stimulation of the autonomic nerve supply of the epididymal fat pad of the rat incubated in an *in vitro* system can result in rapid release of fatty acids and glycerol into the incubation medium.

A recent study using a viral transsynaptic retrograde tract tracer has demonstrated that WAT is innervated by the sympathetic nervous system (11). Several hypothalamic nuclei such as the medial preoptic area and the paraventricular nucleus are identified as origins of efferent sympathetic nerve projection to the WAT (11). Further, it has been reported (12) that the hypothalamic histamine neurons facilitate lipolysis through the activation of sympathetic outflow to the WAT of the epididymis. This report suggests that the histamine neuronal system in the hypothalamus might be involved in activation of sympathetic outflow due to citron

Table I. Effect of Olfactory Stimulation with Grapefruit Oil, Lemon Oil, and Water Vapor on the Mean Discharge Rates of Sympathetic Nerve Innervating the WAT of the Epididymis

Flavor	Control	30 min	60 min	90 min
Grapefruit	68.1 ± 4.5	81.0 ± 7.2[a]	82.9 ± 6.4[a]	104.1 ± 8.4[a]
Lemon	67.8 ± 4.6	80.3 ± 3.6	92.1 ± 6.2[a]	90.2 ± 9.6[a]
Water vapor	60.6 ± 2.5	57.9 ± 1.1	58.0 ± 2.0	59.4 ± 1.6

Note. Each value represents impulses/5 sec ± SEM, $n = 5$.
[a] Significant difference, $P < 0.05$.

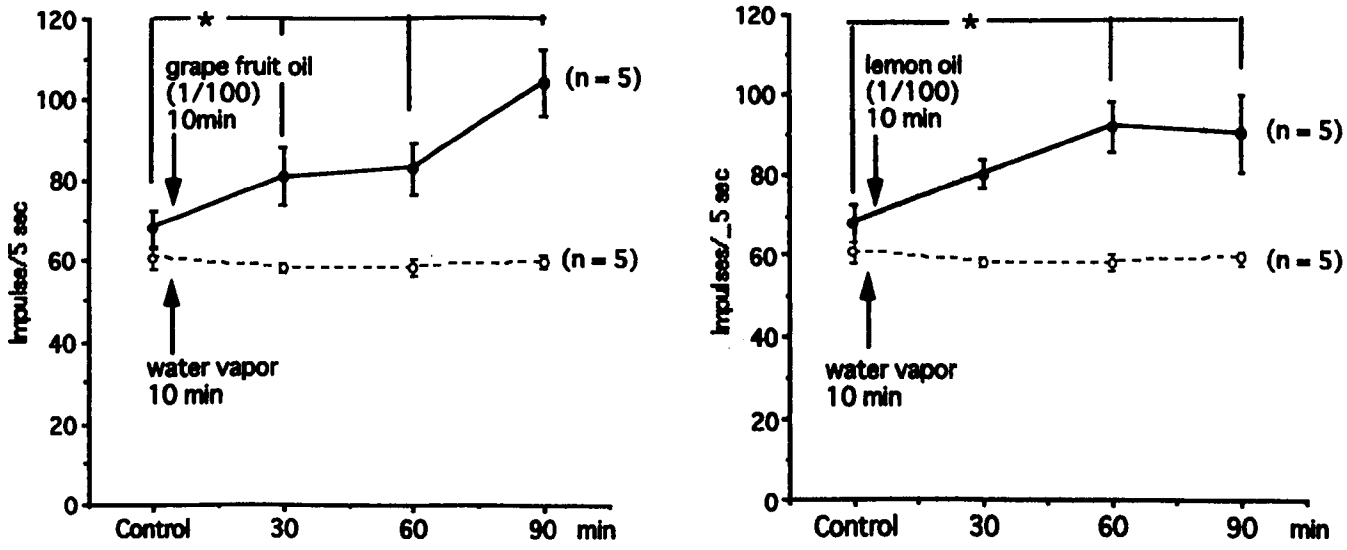

Figure 3. Effect of olfactory stimulation with grapefruit oil (left) and lemon oil (right) on the mean discharge rates of sympathetic nerve innervating the WAT of the epididymis ($n = 5$).

flavor stimulation. Results of the current experiments can be explained by the above-described reports. Thus, it is reasonable to assume that olfactory stimulation with the flavor of the citron group activates sympathetic outflow to the WAT and facilitates the release of norepinephrine from the nerve terminals, which activates lipolysis and finally suppresses body weight gain. Activation of sympathetic outflow to the adrenal medulla following stimulation with the flavor of the citron group was also observed (data not presented). This response suggests an increase in epinephrine release from the adrenal medulla into the circulating blood, which may contribute to acceleration of lipolysis. The effect of the olfactory stimulation with flavors of the citron group on the concentration of fatty acid and glycerol in the blood should be investigated in further study.

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