The effects of odor on cortisol and testosterone in healthy adults

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Abstract

OBJECTIVES: This study aimed to verify the influence of odor on the endocrine system (Cortisol, Testosterone).
MATERIAL AND METHODS: Three odors (Musk, Rose and Floral) and air were given to 16 healthy volunteers (8 males, 8 females), and cortisol (C) and testosterone (T) levels were measured before and after stimulation.
RESULTS: Each odor decreased levels of C, indicating that odor can attenuate stress. In males, T decreased with Musk, but increased with Floral. In females, T significantly increased with Musk, but decreased with Rose and Floral.
CONCLUSIONS: These results indicate that influences on hormone levels depend on the type of odor, and sex differences exist in response to odors.

ORIGINAL ARTICLE

INTRODUCTION

Humans have used odorants since the beginning of history, and customs based on the use of odorants exist in almost all cultures (Van Toller & Dodd, 1988). A variety of studies on putative human pheromones have recently been reported (McClintock, 1971; Stern & McClintock, 1998). Indeed, sense of smell (in terms of odor, pheromone, and others) directly influences the endocrine system and mood, and has been one of the most intensively investigated areas in neuroscience (Laurent, 1999). However, some criticism has been made that scientific studies to identify the underlying mechanisms are lagging behind (Cassileth, 1998). In fact, some reports have physiologically verified the influence of odor on the human body, mostly employing old markers such as electroencephalography (Diego *et al.* 1998; Grosser *et al.* 2000; Van Toller & Dodd, 1988), blood pressure and heart rate (Bensafi *et al.* 2002; Romine *et al.* 1999), and endocrinological markers (hormones) have rarely been employed. In a study on cortisol (C) and odor, Komori *et al.* (1995) reported that citrus odor decreased urine C concentrations in patients with depression. In addition, when subjects taking a stressful test were exposed to the odor of Rose oil, C elevation in blood and saliva was suppressed compared to subjects without such exposure (Tsuchiya *et al.* 2001). Studies have also shown associations between C levels and the ability to distinguish odor. Fleming *et al.* (1997) reported that mothers with high C levels displayed superior recognition of their children's smell. Conversely, the association between testosterone (T), a typical androgen, and sense of smell has been reported. Schneider *et al.* (1958) reported that T increases the threshold of sense of smell in females with sexual dysfunction. In addition, the odor of androstene stimulates secretion of T (Dorfman, 1961). Juette (1995) reported that T levels in the saliva of male subjects increased in an experiment using a fatty acid that was one of the putative human pheromone substances. Furthermore, Monti-Bloch *et al.* (1998) reported that steroid stimulation on the vomeronasal organ (VNO) changed T levels in male subjects. Hirsch and Gruss (1999) reported that stimulation with lavender odor increased blood flow in the genital organs by 30–40% in male subjects. T levels also greatly affect sexual arousal and behavior, aggression, mood and personality (Mazur & Booth, 1998). Further investigation of the effects of odor on testosterone levels is needed to identify the social function and significance of odor and pheromones. This study investigated the biological function of odor with regard to how stimulation with different kinds of odor affects the endocrine system, mainly in terms of attenuation of stress responses, effects on the immune system and associations with sex hormones. In the experiments, 3 kinds of odor (Rose, Floral and Musk) were used as stimulators. The odor of Rose oil and Floral have been shown to attenuate stress in aromatherapy (Buckle, 1997), and Rose oil decreases levels of C, a stress hormone (Tsuchiya *et al.* 2001). Musk has a long history of use as an aphrodisiac in numerous cultures (Van Toller & Dodd, 1988), and is an important substance when considering pheromones in humans. Indeed, some reports have shown that Musk odor affects human psychology (Jacob *et al.* 2002a).

MATERIAL AND METHODS

We recruited university students as volunteer subjects. In selecting subjects, medical conditions that could affect sense of smell (rhinitis, asthma, allergy, etc.) and odor preference were surveyed in a questionnaire, and since smoking decreases olfactory ability (Doty *et al.* 1984), non-smoking was added as a requirement. In addition, before enrolment in the study, sense of smell was examined using a T&T olfactometer (Daiichi-Yakuhin, Tokyo, Japan), and subjects with a normal sense of smell were selected. Internationally established methods to examine sense of smell are lacking (Doty *et al.* 1994), so the T&T olfactometer was used in this study to measure the threshold of sense of smell based on 5 standards. In addition, as conditions for participation, subjects could not be on medicine that affects the endocrine system, drink excessively, or exercise intensively. Furthermore, female subjects had to be those who did not wear perfume regularly and who had regular menstrual cycles (21–35 days in length) without taking contraceptives regularly. After screening tests, 16 healthy college students (8 men, 8 women; mean age, 20.3 years; SD, 1.14 years; range, 18–22 years) were finally selected. Subjects were advised to adopt a similar life cycle (wake-up time, sleeping time and so forth) during the experimental period. One day before the day of the experiments, taking medicine, alcohol intake and stressful activities (intensive exercise, gambling, etc.) were prohibited, and subjects were advised to get sufficient sleep. Likewise, on the day of the experiments, taking medicine, alcohol, caffeine and stimulatory food were prohibited, in addition to use of cosmetics and perfume, and eating and drinking were prohibited from 1 h before the experiments. The female subjects participated in the experiment at the follicular phase.

Experiments were performed during 14:00 to 16:00 in consideration of the diurnal variation of hormones. The room is quiet under artificial lighting with a mean temperature of 18.3 °C. At 1 h before experiments, the room was fully ventilated. One session of the experiment protocol included the following steps: 1) explanation of experiments and acquisition of informed consent; 2) baseline (5 min); 3) collection of saliva (S1: before stimuli); 4) stimulation with odor or air as control (40 min); and 5) collection of saliva (S2: after stimuli). All subjects attended sessions with 4 kinds of stimuli (Musk, Rose, Floral and air). To exclude the effects of time of experiments and sequence of stimuli, all factors were randomized for experiments. All sessions were conducted at 3 days intervals so that the previous session did not affect the following session. During experiments, subjects were instructed to smell the odor while remaining as relaxed as possible and not to sleep. Subjects spilt their saliva into tube. Hormone levels in saliva reflected hormone levels in blood (Khan-dawood *et al.* 1984; Kirschbaum & Hellhammer, 1994), and measurement from saliva samples was a more effective method than blood collection, minimizing psychological and physiological stress such as pain and discomfort. Saliva samples were stored at –20 °C in the freezer immediately after collection, and C and T levels were measured by enzyme immunoassay (EIA) before and after stimulation for comparison. Inter- and intra-assay coefficients of variation were 3.93% and 6.8% for T and 4.1% and 5.6% for C, respectively.

Three odorants of Musk, Rose, and Floral were used as odor stimuli in this study. Each odorant was dispensed by a fragrance expert and fragrances were provided by Kanebo Cosmetics INC. (Kanagawa, Japan). The main constituent of muscone is 3-methyl-cyclopentadecalacton. The major constituents of Rose oil are l-citronelle, nerol and geraniol. The major constituents of Floral are beta-phenylethylalcohol, linalool and methyl dihydrojasmonate. Filter paper was soaked in up to 0.03 ml of each of the 3 fragrances and placed in a glass bottle, and the air was saturated with the fragrance. Odorless air flowed through activated charcoal at a rate of 1 L/min. The strength of scent was unified in each section of the experiments, and flow of air with odor was maintained at a fixed rate. The distance between outlet of odor and the nostrils was fixed at 10 cm.

RESULTS

Mean C level was 0.060µg/dl (range, 0.010–0.173 µg/dl) in males and 0.371 µg/dl (range, 0.117–0.806 µg/dl) in females. To investigate the effects of stimuli (Musk, Rose, Floral and Control) and sex differences on C level, Analysis of variance (ANOVA) was conducted using odors and sex as factors. As a result, the main effects of C ($F(1,55)=7.903$, $p=0.0068$) and sex ($F(1,55)=156.423$, $p<0.0001$) were significant. A significant trend of interaction was identified between C changes and sex ($F(1,55)=3.554$, $p=0.0647$)(Figure 1; Figure 2). In addition, C for both sexes decreased with all odors, although the change in female subjects was more significant.

Mean T level was 92.253 pg/ml (range, 46.046–132.747 pg/ml) in males and 64.571 pg/ml (range, 14.916–169.137 pg/ml) in females. To investigate the effects of stimuli (Musk, Rose, Floral and Control) and sex difference on T level, ANOVA was used on changes in T, with odor and sex as factors. As a result, the main effect of sex was significant ($F(1,54)=9.118$, $p=0.0039$) and a significant interaction was existed between odors and T changes and sex ($F(3,54)=3.344$, $p=0.0257$)(Figure 3; Figure 4). T was significantly higher in males than in females. Musk decreased T in males, but markedly increased T in females. Rose decreased T in female subjects. Floral increased T in males, but decreased T in females.

DISCUSSION

In this experiment, Musk, Rose and Floral decreased C in both sexes. Tsuchiya *et al.* (2001) suggested that the odor constituents in Rose decreased C levels and exerted a stress-reducing effect, and the present results indicate a possible sex difference in the stress-reducing effects of Rose odor. Our results support previous reports that odor can have stress-reducing effects. Moreover, the existence of sex differences in C were strongly indicated. Sex differences in the effect of odors appear likely, although further investigations are required. In male subjects, T decreased with Musk, but increased with Floral. In female subjects, T decreased with Rose and Floral, but increased significantly with Musk. T is well known as a hormone that greatly affects human sexual behavior and is associated with sexual arousal (interest, desire, etc.) (Alexander *et al.* 1997; Stoleru *et al.* 1993; Mazur & Booth, 1998), and has been shown to affect the frequency and manner of sexual behavior (Knussmann *et al.* 1986; Hirschenhauser *et al.* 2002). Furthermore, T is closely related to aggressiveness in animals, including humans (Dabbs *et al.* 1995: Archer, 2006). The finding that Musk decrease T in male indicates the possibility that these odors function to reduce sexual desire and aggressive behavior. Conversely, the increase in T with Floral suggests activation of sexual behavior. Actually, Hirsch and Gruss (1999) reported that lavender a kind of floral increases blood flow in male genitals and improves sexual function in males. Floral might thus affect sexual behavior in males. In our study, Musk increased T in females. In fact, the odor of Musk (androstenol) has been noted to intensify sexual arousal in females (McCollough *et al.* 1981). Based on this, we speculate that Musk functions to stimulate sexual activity in females. The present results show that Musk exerts different effects on T by sex, and further investigation is thus required into the function of Musk. The results in this study indicate that different kinds of odor affect different hormones and changes in hormone levels differ by sex. In addition, odorants affect

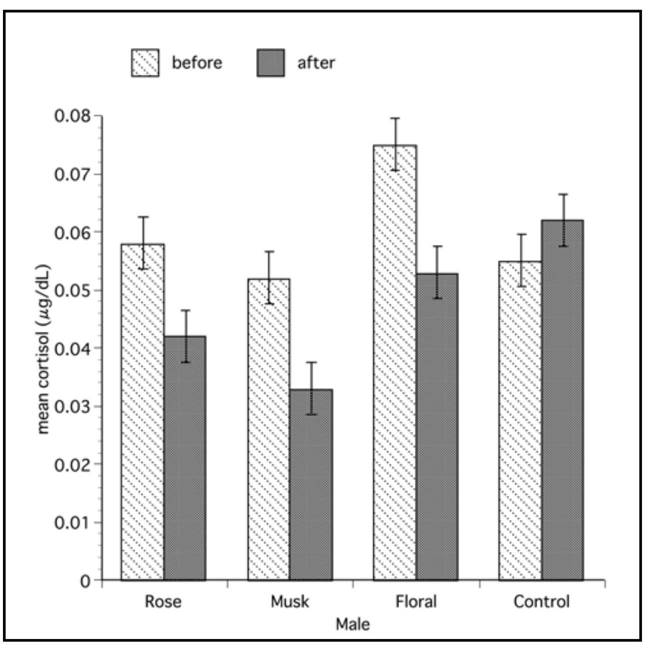

Figure 1. Cortisol levels in male subjects. (Cortisol decreased in all odor conditions).

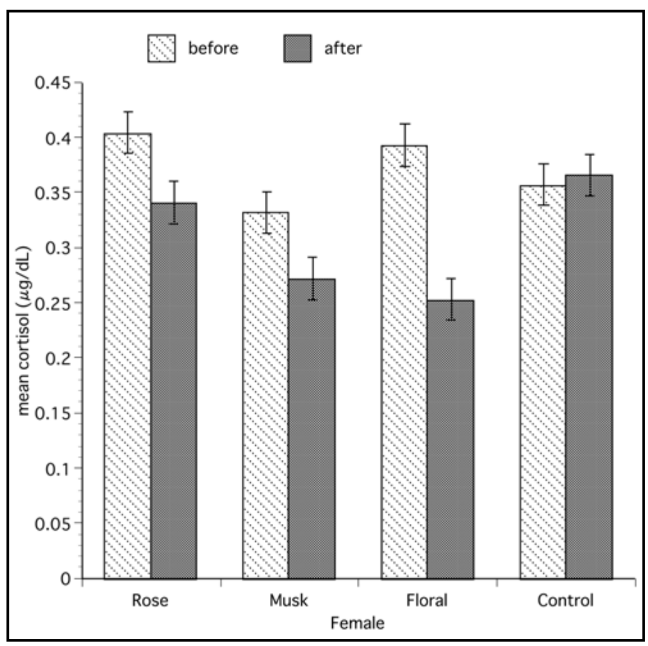

Figure 2. Cortisol levels in female subjects. (Cortisol decreased in all odor conditions).

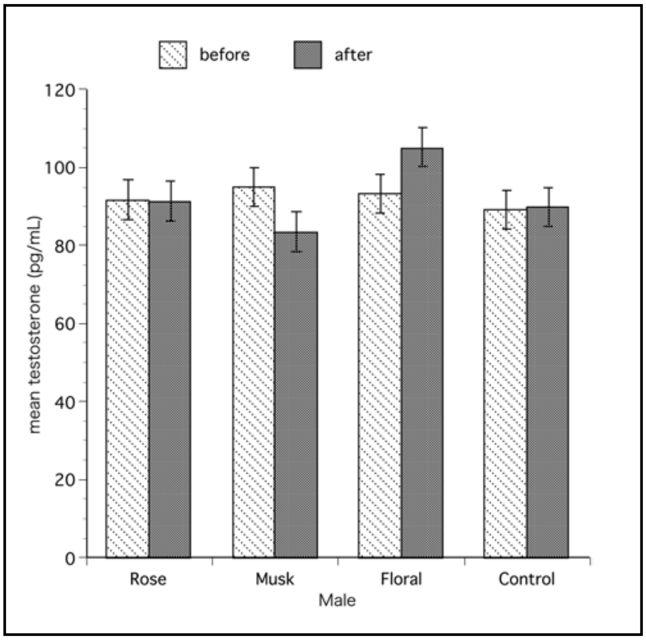

Figure 3. Testosterone levels in male subjects. (Testosterone increased in the Floral group though testosterone reduced in the Rose and the Musk group).

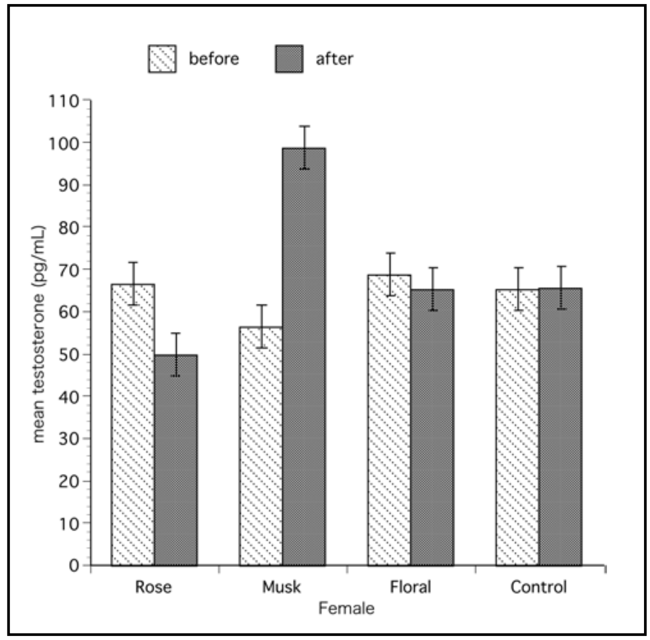

Figure 4. Testosterone levels in female subjects. (Testosterone increased in the Musk group though testosterone reduced in the Rose and the Floral group).

sex hormones, reduce stress and influence the immune system. Although odor has been noted to greatly affect the human endocrine system, few endocrinological studies have been reported on odor. Future studies should focus on various factors related to sense of smell, such as individual differences in sense of smell (Doty *et al.* 1981), and the relationship between menstrual period and sense of smell in female (Benton, 1982; Morofushi *et al.* 2000; Pause *et al.* 1996; Purdon *et al.* 2001) to accumulate data on relationships between odor and the endocrine system. Humans have been shown to use odorants as a non-linguistic communication tool and odor greatly affects human social behavior and the psychological and physiological state. In fact, the notion that sense of smell directly affects the endocrine system and feelings more than any of the other human senses has attracted the attention of neuroscientists, and studies on the effects of odorants on humans could offer important information to elucidate the mechanisms of human feelings. Humans are overwhelmingly inferior to other animals in terms of olfactory sensitivity, but odor has a greater effect than we think. We can speculate that odorants including pheromones have played various functions in the social development of humans (Ackerl *et al.* 2002; Kohl et. al. 2001; Grammer, 1993; Kohl & Francoeur, 1995; Jacob et. al. 2002b; Thornhill & Gangestsd, 1999). Endocrinological studies on the effect of odor on humans would be important when investigating evolutionary function and its significance, and further investigation is expected.

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