

Positive Relationship between Menstrual Synchrony and Ability to Smell 5 α -Androst-16-en-3 α -ol

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Abstract

To explore the possibility that compounds which were identified as pheromones in experimental animals mediate human menstrual synchrony, we examined the relationship between menstrual synchrony and the ability to smell putative pheromones, 5 α -androst-16-en-3 α -ol (3 α -androst-enol) and 5 α -androst-16-en-3-one (5 α -androst-enone). When we examined menstrual synchrony among 64 women living together in a college dormitory, we found that 24 (38%) of them became synchronized with room-mates in 3 months. Afterwards, dilution series of 3 α -androst-enol and 5 α -androst-enone and the control odorant (pyridine) were presented to the 64 women and sensitivity to the odors was compared between synchronized and non-synchronized women. No difference was found between the two groups of women in the detection threshold for pyridine, indicating that general olfactory ability did not differ between them. The detection threshold for 3 α -androst-enol of synchronized women was significantly lower than that of non-synchronized women, but no difference in the threshold for 5 α -androst-enone was found between synchronized and non-synchronized women. These results indicate that the women who showed menstrual synchrony had a higher sensitivity to 3 α -androst-enol but not necessarily to 5 α -androst-enone.

Introduction

McClintock (McClintock, 1971) has shown that menstrual cycles of college students become synchronized when they live together as room-mates. Since then many studies have confirmed menstrual synchrony between women, i.e. close friends, mothers and daughters and co-workers [reviewed by Weller and Weller (Weller and Weller, 1993)]. In each of these studies, women who spent the most time together were most likely to show menstrual synchrony. It has been demonstrated that odors emanating from the axillary region may mediate these effects (Preti *et al.*, 1987; Russel *et al.*, 1980; Stern and McClintock, 1998), but the active principles in the axillary sweat have not yet been identified.

At least two odorous steroids are known to be secreted from axillae, 5 α -androst-16-en-3-one (5 α -androst-enone) and 5 α -androst-16-en-3 α -ol (3 α -androst-enol) [reviewed by Gower and Ruparelia (Gower and Ruparelia, 1993)]. 5 α -Androst-enone has a urine-like smell or a sandalwood odor (Amoore *et al.*, 1977). 3 α -Androst-enol has a musk-like smell or a floral odor (Labows and Wysocki, 1982). In pigs, these two steroids have been demonstrated to act as pheromones. They are secreted in the saliva of the boar and act as a releaser pheromone effective in eliciting the characteristic immobilization response of the estrous sow to the advance of its mate (Perry *et al.*, 1980). A possible contribution of these odorous steroids to human interpersonal relationships

has also been suggested by several laboratories (Kirk-Smith *et al.*, 1978; Gustavson *et al.*, 1987; Cowly and Brooksbank, 1991).

Most mammals have two olfactory systems, the main system, which receives sensory inputs from the olfactory mucosa and connects to the rest of the central nervous system via the main olfactory bulbs, and the accessory system, which receives inputs from the vomeronasal organ and connects to other centers in the brain via the accessory olfactory bulbs (Scalia and Winans, 1976). In both systems there are pathways from the olfactory bulbs to the hypothalamus, the center controlling the secretion of luteinizing hormone. The accessory system mediates the pheromonal effect in rodents [reviewed by Marchewska-Koj (Marchewska-Koj, 1984)]. However, it appears that the main olfactory system plays a major role in the pheromonal effect in ewes (Martin *et al.*, 1986) and pigs (Dorries *et al.*, 1997). If pheromones that mediate menstrual synchrony use the main olfactory system, a causal relationship between ability to smell a pheromone and a possible role of the pheromone in mediating synchrony can be suggested by comparing the ability to smell the pheromone between synchronized and non-synchronized women. In the present study we have examined the relationship between menstrual

synchrony and the ability to smell the putative pheromones 3 α -androsthenol and 5 α -androsthenone.

Materials and methods

Subjects and procedure

We examined menstrual onset dates in 67 Japanese women living together in a college dormitory. We selected the women who had had regular menses (26–32 days) for the three previous cycles, had not been pregnant or had not taken oral/hormonal contraceptives, had taken no medication regularly and had no mental or gynecological disorders. They were living in 18 triple or quadripartite rooms. The age of subjects ranged from 18 to 23 (mean \pm SD 19.1 \pm 1.0) years old. These subjects started to live together as room-mates in April.

From January through July each subject recorded the menstrual onset date. The experiments were terminated 3 months after living together because most subjects left the dormitory for home vacations in August. Menstrual onsets of a person were compared with those of the other room-mates in each room and for each person we chose the room-mate whose onset date in July was closest to that of the person, as the partner in a pair.

Subjects were classified into two groups, synchronized and non-synchronized, according to the criteria used by Weller and Weller (Weller and Weller, 1993, 1997). We calculated the average menstrual cycle length from January through March. Mean cycle length divided by four was used as the cut-off point. The range of onset differences for women with a 28 day cycle was between 0 and 14 days; 7 days (cut-off point) was considered the middle point of no effect. Onset differences of <7 (0–6) days indicates synchrony and >7 (8–14) days indicates non-synchrony. As synchronized women we defined those whose menstrual onset difference from the partner was longer than the cut-off point in January through March and shorter than the cut-off point in June and July. We excluded three persons from this study whose lengths of menstrual cycle did not change during this study even though the difference in the onset of menstruation was shorter than the cut-off point, because we could not determine whether these women became synchronized with the partner or not after April. Women who did not satisfy the above criteria were classified in the non-synchronized group.

On 1–7 August subjects were asked to indicate their age at menarche and their usual length of menstrual flow. Subjects were also asked to rate, on a two-point scale, how involved they felt with their partners in the pair (2, close relationship; 1, poor/not close relationship). The answers to these questions were compared between synchronized and non-synchronized women.

Stimuli and sensory testing

On 1–7 August we determined the detection threshold to

odors. The sensory tests were not standardized or controlled for the phase of menstrual cycle because there were no changes in the sensitivity to pyridine, 5 α -androsthenone and 3 α -androsthenol during phases of the menstrual cycle (unpublished data). Stimuli and sensory testing was conducted in a quiet room to aid concentration on the test. Subjects were asked not to use perfume during the experiments. The subject's eyes were closed to preclude the possibility of corneal trigeminal stimulation (Doty *et al.*, 1978).

First of all subjects were screened for general olfactory capacity. Polypropylene tubes (2.0 ml) with pop-top caps were used to present stimuli. A single concentration (0.1% in odorless light white mineral oil) of phenylethyl alcohol (PEA) in the tube was given after the blank tube (vehicle only). The subjects who could not smell this odor were excluded from the present study as showing total anosmia. This odorant was chosen because it apparently cannot be detected by people who are totally anosmic but who possess an intact trigeminal sense (Doty *et al.*, 1978).

Next, we presented eight different concentrations of pyridine ranging from 2.91 μ M (step 1) to 0.372 mM (step 8), each in 1.5 ml of mineral oil. We used pyridine because this odor was successfully used in similar experiments to measure the olfactory detection thresholds of patients (Sherman *et al.*, 1979). Complete concentration series for pyridine were obtained by binary serial dilution from the most concentrated stimulus. These concentrations were thought to be below the threshold for trigeminal stimulation (Doty *et al.*, 1978). Each concentration tube was paired with the blank tube and the order of presentation (odor or blank) was randomized. Detection thresholds were determined with four ascending concentration series. Subjects were asked to indicate which tube was the odorized one. A series was terminated when a person correctly identified the odorized tube in four odor versus blank trials, the lowest value of the four being designated the threshold.

5 α -Androsthenone and 3 α -androsthenol were tested in a manner similar to pyridine except for drug concentrations. We used 10 concentrations of 5 α -androsthenone (Sigma) and 3 α -androsthenol (Sigma), ranging from 5 μ M (step 1) to 5 mM (step 10) in 1.5 ml of mineral oil. Complete concentration series for 5 α -androsthenone and 3 α -androsthenol were obtained by 2.16 times serial dilution from the most concentrated stimulus. In any series, individuals failing to detect each odorant at step 10 were assigned a threshold value of 11.

The protocol observed the tenets of the Helsinki Declaration and was approved by the Ethics Committee at Yokohama City University.

Statistical analysis

Thresholds were subjected to non-parametric analyses because the data were not normally distributed. The absolute values of threshold differences were determined for each group (synchronized or non-synchronized) and analyzed

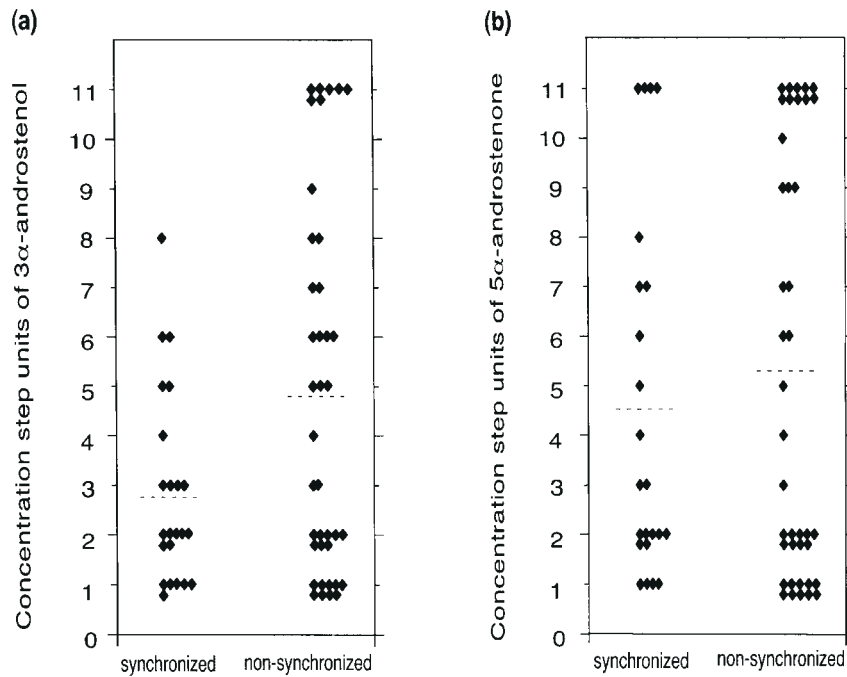


Figure 1 3 α -Androstenol (a) and 5 α -androstenone (b) detection thresholds for each of the synchronized and non-synchronized women. Concentrations of both drugs ranged from 5 μ M (step 1) to 5 mM (step 10). Complete concentration series for the drugs were obtained by 2.16 times serial dilution from the most concentrated stimulus. Dotted lines indicate averages of drug detection thresholds.

with the Mann–Whitney test of ranks. Statistical significance was set at $P < 0.05$.

Results

According to our criteria for menstrual synchrony, we excluded three women from this study whose menstrual cycles did not change during this experiment. In the rest of the individuals the date of menstrual onset was compared with those of room-mates. Twenty-four (38%) out of 64 women synchronized with room-mates. The time since menarche was 7.1 ± 1.8 years in synchronized women and 7.4 ± 1.2 years in non-synchronized women. The length of menstrual flow was 5.4 ± 1.6 days in synchronized women and 5.7 ± 1.1 days in non-synchronized women. The score for quality of relationship with the partner of the pair was 1.5 ± 0.5 in synchronized women and 1.6 ± 0.5 in non-synchronized women. There were no differences in these items between synchronized and non-synchronized women.

There was one non-synchronized woman that could not smell PEA. She was judged to be totally anosmic and excluded from further study. There was no difference between synchronized and non-synchronized women in the detection threshold for pyridine (data not shown). This indicates that general olfactory ability did not differ between the two groups. The detection threshold for 3 α -androstenol was significantly lower in synchronized women than in non-synchronized women (Figure 1, $P < 0.01$). No significant difference between the two groups was observed in the threshold for 5 α -androstenone (Figure 1).

Some subjects failed to detect both 3 α -androstenol and 5 α -androstenone, even when presented with the strongest concentrations (Table 1). All the women who were synchronized could detect 3 α -androstenol but not necessarily 5 α -androstenone.

Discussion

In the present study we have demonstrated that the menstrual cycle in 24 (38%) out of 64 women became synchronized with that of room-mates in 3 months. There were no differences between synchronized and non-synchronized women in the time since menarche, the length of menstrual flow and quality of relationship with the partner of the pair. However, synchronized women had a higher olfactory acuity to 3 α -androstenol, compared with non-synchronized women, although no difference was observed between the two groups in the sensitivity to pyridine and 5 α -androstenone. These results suggest that the ability to perceive the odor emitted by 3 α -androstenol may be related to menstrual synchrony.

Sensory perception of 3 α -androstenol and 5 α -androstenone exhibited great individual variation. The detection threshold was continuously distributed in individuals who could smell these steroids. The other individuals failed to detect the odor even when presented with the strongest concentration. The rate of anosmia for 5 α -androstenone in the present study was lower than that in other reports (Amoore *et al.*, 1977; Labows and Wysocki, 1982). This difference may be due to a racial difference (Gilbert and

Table 1 Mean detection thresholds and rates of anosmia

| | 5 α -Androstenone | | 3 α -Androstenol | |
|-----------------------------------|--------------------------|---------------------|-------------------------|---------------------|
| | Mean threshold | Rate of anosmia (%) | Mean threshold | Rate of anosmia (%) |
| Synchronized (<i>n</i> = 24) | 4.6 \pm 3.7 | 17.4 | 2.9 \pm 1.9 | 0 |
| Non-synchronized (<i>n</i> = 39) | 5.3 \pm 4.2 | 25.0 | 4.8 \pm 3.7 | 17.5 |
| | <i>Z</i> = 0.27 | | <i>Z</i> = 1.71 | |
| | <i>P</i> > 0.40 | | <i>P</i> < 0.05 | |

Z scores were obtained by Mann–Whitney comparison. Mean threshold is in concentration step units.

Wysocki, 1987). Furthermore, it is noteworthy that all the women who synchronized could detect 3 α -androstenol but not necessarily 5 α -androstenone. This supports the above speculation that the ability to smell 3 α -androstenol is related to menstrual synchrony. However, some non-synchronized women showed a high sensitivity to 3 α -androstenol and the rate of 3 α -androstenol anosmia was not so high. It is possible that these non-synchronized women sensitive to 3 α -androstenol would become synchronized if they lived with room-mates for longer than 3 months because the menstrual cycles of college room-mates become increasingly synchronized over a period of 4 months (McClintock, 1971).

3 α -Androstenol seems to be synthesized by microbiological modification of odorless substances originally present in apocrine secretions (Leyden *et al.*, 1981). The axillary 3 α -androstenol levels in women show menstrual variation; the highest concentration of this compound is produced in the mid-follicular phase, prior to ovulation (Preti *et al.*, 1987). Recently it has been shown that axillary compounds from women in this phase of the menstrual cycle shorten the time to ovulation and length of the menstrual cycle, whereas in the ovulatory phase they lengthen them (Stern and McClintock, 1998). Therefore, 3 α -androstenol is a possible pheromone included in axillary compounds secreted in the follicular phase.

We assume that the pheromones that mediate the menstrual synchrony in the present study were detected by the main olfactory system, as shown in some animals (Martin *et al.*, 1986; Dorries *et al.*, 1997). It is, however, possible that the pheromonal effect is mediated by the accessory olfactory system, since axillary compounds which change the length of the menstrual cycle seem to be below the olfactory threshold (Stern and McClintock, 1998). We should examine the effect of 3 α -androstenol on the length of the menstrual cycle before we suggest that 3 α -androstenol is involved in menstrual synchrony.

It seems that synchrony between two women could be achieved by either one or both of them shifting their cycles to synchronize with the other. It is possible that the ability to smell pheromones correlates only with a woman's likelihood of shifting her own cycle in response to another woman's pheromones and not with a woman's likelihood of causing a

cycle shift in another woman. Even if only one partner of a pair changed their menstrual cycle, both of the pair would be judged to be synchronized according to the criteria of the present study. In the present study, however, this was not the case: either both partners of a pair or neither of them changed their cycle.

(*Z*)-7-Dodecen-1-yl acetate is used as a pheromone by females of >126 species of insects and the elephant (Rasmussen *et al.*, 1996). As for 3 α -androstenol, effects on women's mood (Benton, 1982) and sexual arousal (Gustavson *et al.*, 1978; Cowly and Brooksbank, 1991) have been reported, so that 3 α -androstenol may also not be species specific. In addition to these effects, a new role for 3 α -androstenol in menstrual synchrony is indicated by the present study.

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