Individual Differences in Sensitivity to the Odor of 4,16-Androstadien-3-one

Johan N. Lundström[1], Thomas Hummel[2] and Mats J. Olsson

[1]Department of Psychology, Uppsala University, Uppsala, Sweden and [2]Smell & Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany

Correspondence to be sent to: Johan N. Lundström, Department of Psychology, Uppsala University, Box 1225, SE-751 42, Uppsala, Sweden. e-mail: johan.lundstrom@psyk.uu.se

Abstract

Individual differences in sensitivity to the putative human pheromone androstadienone were investigated in three experiments. In experiment 1, the absolute detection threshold for androstadienone was determined to be 211 μM using the method of constant stimuli. Detection for the related compound estratetraenol was also investigated but a threshold could not be determined. In experiment 2, using an adaptive threshold test on 100 participants, the sensitivity distribution for androstadienone, but not for the reference odor phenylethyl alcohol, was bimodal, with a smaller group of individuals with a high sensitivity to androstadienone (supersmellers). A lack of correlation between thresholds for androstadienone and phenylethyl alcohol further suggested that the bimodality for androstadienone was not due to individuals with a high general olfactory sensitivity. In line with an earlier observation, there was a statistical tendency for women to be more sensitive to androstadienone than men. Results of experiment 3 preclude the possibility that the bimodal sensitivity distribution for androstadienone would depend on individual differences in trigeminal activation. Altogether, the current study suggests that olfactory sensitivity to androstadienone is bimodally distributed in the population with a subgroup consisting of highly sensitive people.

Key words: bimodality, pheromone, supersmellers, threshold

Introduction

Research on human chemosignals (so-called pheromones) has recently focused on the steroids 4,16-androstadien-3-one (androstadienone) and 1,3,5(10),16-estratetraen-3-ol (estratetraenol). It has been reported that androstadienone affects subjects' mood (Jacob and McClintock, 2000; Lundstrom *et al.*, 2003), psychophysiological responses (Grosser *et al.*, 2000; Jacob *et al.*, 2001a) and brain activity (Jacob *et al.*, 2001b; Savic *et al.*, 2001). Androstadienone is a member of the family of odorous 16-androstenes and can be found in the peripheral blood plasma of men to the extent of 98 ng/100 ml blood (Brooksbank *et al.*, 1972) and in their axillary secretion with a mean value of 228 pmol/total axillary hair weight (Nixon *et al.*, 1988). Androstadienone can also be found in women, but at a much smaller concentration (Brooksbank *et al.*, 1972). Male axillae are said to be dominated by coryneform bacteria, while women's axillae microflora are said to be dominated by micrococcacea bacteria (Jackman and Noble, 1983). Although examined in only a few subjects, it has been shown that there is a high correlation between the presence of coryneform bacteria and the amount of 5α-androst-16-en-3-one (androstenone) (Gower and Ruparelia, 1993). Based on this, it has been proposed that androstadienone may be created from androstadienol

by axillary coryneform bacteria and is later transformed into the more odorous androstenone (Mallet *et al.*, 1991; Rennie *et al.*, 1991). It is therefore conceivable that androstadienone may be a precursor to many of the other 16-androstenes (Gower and Ruparelia, 1993). Compared to androstadienone, the steroid estratetraenol has been investigated to a lesser degree. Estratetraenol has been found to affect subjects' mood (Jacob and McClintock, 2000), psychophysiological recordings (Jacob *et al.*, 2001a) and brain activity in males (Savic *et al.*, 2001). Estratetraenol is a compound that is structurally similar to estrogens. However, with regard to being a compound of human origin, estratetraenol has only been isolated from urine of pregnant women in the third trimester (Thysen *et al.*, 1968).

An interesting aspect of the notion of putative human pheromones relates to the question whether they exert their effects due to conscious experience of the exposure or not. It has been clearly shown that consciously perceived, non-pheromonal odorants can modulate behavior (Baron, 1988). Until today, studies on potential putative human pheromones have failed to determine to what extent the compound in question could be consciously experienced (Benton, 1982; Benton and Wastell, 1986; Cutler *et al.*, 1998; Thorne *et al.*,

2002). Surprisingly, none of the published studies on potential putative human pheromones has used proper psychophysical testing to ascertain that the concentration of the putative pheromone in question is not above detection threshold, or that control odors are not discernible from the investigated putative pheromones.

One paper (Gower and Ruparelia, 1993) has been cited as a reference to the absolute threshold value of androstadienone. However, this reference is referring back to an article that investigated the olfactory threshold of androstenone and not androstadienone (Patterson and Griffiths, 1969). To our knowledge, there is only one peer-reviewed study aiming at the determination of androstadienone thresholds in humans. Koelega and Köster (1974) investigated potential sex differences in odor perception of several biological (odorants of human origin) and non-biological odors, including androstadienone. They reported that women had a generally higher sensitivity to biological odors than men and that there was a significant sex difference in the threshold for androstadienone where women had a threshold of 11.4 mM and men 19.5 mM. However, as the authors pointed out correctly, the method used in the study was acceptable in terms of determination of sex differences but was flawed with regard to determination of absolute thresholds.

The present paper reports results of three experiments on individual differences in androstadienone sensitivity. Since it is important to consider the contribution of consciousness when studying the effects of putative human pheromonal compounds, we conducted a study (experiment 1) with the aim of determining the absolute threshold for the two steroids androstadienone and estratetraenol. In experiment 2, we measured the threshold for androstadienone and the compound phenylethyl alcohol with a broader stimulus range than used in experiment 1 with the aim of assessing the potential bimodal sensitivity distribution of androstadienone. Finally, experiment 3 investigated potential trigeminal activation of the putative pheromonal compound androstadienone.

Experiment 1

Method

Participants

The experiment involved 42 healthy volunteers (22 women) with a mean age of 26.2 years (± 5.9 years). None of the participants reported nasal surgery. All subjects rated themselves as normosmic without any nasal problems. They were recruited through posters on the university campus and were rewarded with either a cinema voucher or course credits.

Materials and compounds

Androstadienone and estratetraenol were obtained from Steraloids Inc. with a purity of ≥98% (verified by Steraloids Inc.). The active compounds were dissolved in propylene glycol (purity ≥99%; VWR International), a relatively odorless and non-toxic liquid. Five concentrations of both androstadienone and estratetraenol were established using dilution steps with a 2.5-increase of liquid concentration (androstadienone: 76–3000 µM; estratetraenol: 192–7500 µM). These step sizes were based on a pilot study ($n = 8$) indicating that estratetraenol was not detectable at concentrations below 3000 µM. Two identical stimulus sets were prepared for each odorant. Each set consisted of the five concentrations mentioned above and one blank (referred to as target) each of which was pared with two controls (referred to as lure). The stimuli were presented from 250 ml polypropylene squeeze bottles with pop-up spouts. These were washed and air-dried before use in order to minimize the background odor, and filled with 15ml of the solution.

Procedure

Thresholds for androstadienone and estratetraenol were measured on two different occasions in counterbalanced order using similar procedures. Threshold tests were of a three-alternative, forced-choice design using one target and two control bottles placed on a table at three fixed positions. The presentation order was randomized with regard to the presented concentration of the target and the position on the table. When all six concentrations of the stimuli had been presented once in the first set, the other set was used (and so forth) in order to allow the headspace in the bottles to saturate. Further, this use of two different sets also ensured that no single bottle would be presented twice successively. All three bottles of the individual stimuli were placed in front of the participant on marked, numbered, positions on the table and they were asked to sniff each bottle just once, in the order of presentation, by holding the bottle just below the nose and gently squeezing it once. They were told that one bottle in each trial was different and that they should pick the 'odd' one. No feedback was provided to the participants with regard to correct identification of the odor. After making a decision they were asked to rate the confidence in their choice on a labeled scale ranging from zero (absolutely uncertain) to five (absolutely certain). This continued until all six stimulus levels had been presented nine times each, summing up to a total of 54 trials. Before the test started, training trials with three blank bottles containing only propylene glycol were conducted in order to get the participants acquainted with the test procedure. Participants had a 5 min pause outside the testing facility in the middle of each session to counteract sensory adaptation; each session took ~45 min.

Data treatment and threshold calculation

For each participant and concentration, the proportion of correct responses was calculated. The proportion of correct responses for each individual was plotted against log odor concentration which yielded an ogive function. Logistic functions were fitted to the data and 66.6% thresholds were

determined. Analyses of variance (ANOVA) were used to statistically test for group differences in threshold values.

Results and discussion

The absolute threshold for androstadienone was 211 μM, expressed as concentration in solution (see Figure 1). The female participants had an absolute threshold value of 168 μM and the male participants 251 μM, which is a nonsignificant difference [$F(1,39) = 0.42$].

The threshold value obtained in this experiment for androstadienone is considerably lower than the threshold value previously reported by Koelega and Köster (1974). There may be several reasons for this discrepancy. The technique used by Koelega and Köster, as correctly stated by the authors, was not optimized for determination of absolute threshold values. Both stimulus presentation and preparation were different, which is known to have impact on individual thresholds (Pierce *et al.*, 1996).

It was not possible to determine the detection threshold for the stimulus range of estratetraenol concentrations used in this study. None of the participants was able to detect estratetraenol at its highest concentration (7500 μM). This finding corroborates earlier observations indicating that estratetraenol is an odorless chemical compound (Koelega and Köster, 1974; Ohloff *et al.*, 1983; Prelog *et al.*, 1945). Unfortunately, these studies do not specify the dilution range used. However, Savic *et al.* (2001) state that crystalline estratetraenol has an odor which implies that the absolute threshold value for estratetraenol probably lies well above the highest dilution used in this study.

Previous studies on potential human pheromones focused on women not taking oral contraceptives. The present study yielded no evidence that the use of oral contraceptives ($n = 12$) affected olfactory sensitivity for androstadienone [$F(1,19) = 0.90$, n.s.].

The most common finding regarding confidence judgments in sensory discrimination tasks has been the underconfidence phenomenon (Bjorkman *et al.*, 1993; Juslin *et al.*, 1998), where the mean probability of being correct assumed by the participant, X, falls short of the actual proportion correct discriminations, C. Confidence is commonly measured by this difference, $X - C$, and expressed as an *O*ver- or *U*nderconfidence score, *O/U*. The participants are said to be calibrated to the extent that the proportion correct in each confidence category matches the subjective probability of that category. Participant's confidence judgments in this experiment were well calibrated with regard to their actual performance ($O/U = -0.03$). As stated above, participants are often underconfident in most sensory tasks. However, the participants in this study were not. Conscious experience and behavioral discrimination concurred. This implies that the use of verbal descriptors for measurement of discriminatory performance between the test and control stimuli employed, among others, by Jacob and colleagues in their studies (Jacob *et al.*, 2001a; Jacob and McClintock, 2000), could be accurate measurements of conscious discriminatory performance. However, verbal discrimination puts high demands on memory and accuracy of verbal descriptors (White, 1998). Therefore, a discriminatory test where participants are able to make a direct comparison between test and control solution may still be recommended if the aim of the study is to present solutions that cannot be discriminated by the participants. A suggestion of such a test has been made elsewhere (Lundstrom *et al.*, 2003). When performance on each concentration level was considered, we observed that some participants at the lowest stimulus level (76 μM) could clearly sense the concentration easily and consequently expressed a high level of confidence. This finding corroborated earlier findings that some participants show a high sensitivity to androstadienone (Lundstrom *et al.*, 2003). However, whether these highly sensitive 'supersmellers' really had a specific sensitivity for androstadienone or whether they just had a generally high olfactory acuity was not clear. Another question concerns whether the existence of supersmellers indicates a bimodal sensitivity distribution or created by a cutoff effect of the normal distribution curve.

To summarize: the absolute threshold for androstadienone is 211 μM. The relation between participants' sensory discrimination and conscious perception seemed to be well calibrated. Finally, it was not possible to determine an absolute threshold for estratetraenol for the range of concentrations used.
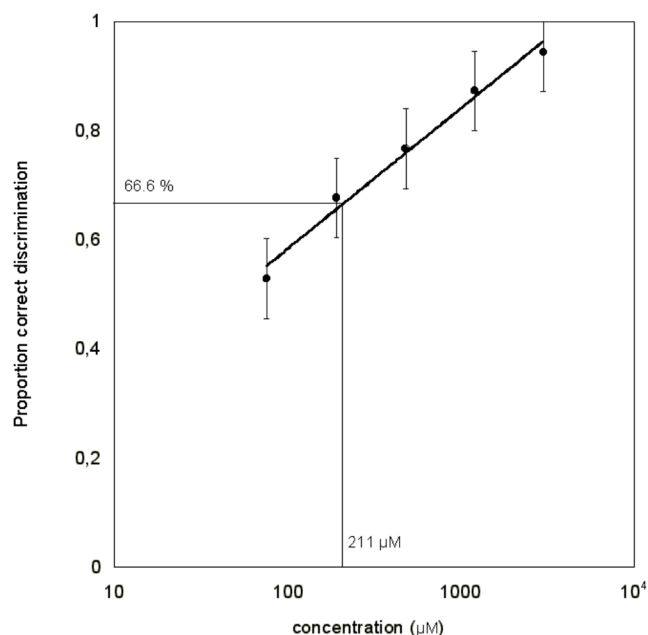
Figure 1 Relationship between proportion correct responses and androstadienone stimulus concentration, in micromolar concentrations (log). Error bars represent SEM.

Experiment 2

Extensive psychophysical work has been done on the related steroid androstenone. Several studies have shown that many normal adults cannot detect the characteristic urinous odor of androstenone. Around 40–50% of the population has a low sensitivity/specific anosmia towards androstenone (Labows and Wysocki, 1984; Wysocki and Beauchamp, 1991), which appears to have both a genetic (Wysocki and Beauchamp, 1984) and a developmental component (Dorries et al., 1989). As androstadienone probably is a precursor to androstenone, it is conceivable that a similar bimodality exists for the olfactory sensitivity to androstadienone. However, the results of experiment 1 suggested that there are two sensitive groups, one smaller group that is more sensitive and one larger that is less sensitive. Bimodality of sensitivity composing clearly sensitive groups has been reported in the literature before for the odors of methylmercaptan and acetone (Lison et al., 1980; Odeigah, 1994).

The first aim of experiment 2 was to investigate whether there is a bimodal sensitivity distribution for androstadienone thresholds. The second aim was to correlate individual sensitivity to androstadienone with sensitivity to phenylethyl alcohol in order to investigate whether potential supersmellers are specifically sensitive to androstadienone or whether they have a higher olfactory sensitivity in general.

Method

Participants

In experiment 2, 100 people participated, with a mean age of 24 ± 3.9 years (58 women: mean age 24 ± 4.2; 42 men: mean age 24 ± 3.5). They were all recruited through posters on the campus. Inclusion criteria were self-reported absence of nasal congestion, infection or decreased olfactory function at the time of the testing. Twenty-six participants had some form of allergy but showed no signs of this at the time of testing; 13 participants labeled themselves as smokers. Having an allergy or using tobacco products did not have a significant effect on either phenylethyl alcohol (all $t < 0.41$, all $P > 0.34$), or androstadienone thresholds (all $t < 1.57$, all $P > 0.12$). Participants were asked not to drink anything other than water, not to use any form of tobacco products for 1 h before testing and not to wear perfume during the experiment. After completion of the testing, participants were rewarded with a cinema voucher.

Material and compounds

Two different olfactory threshold tests were used to assess participants' olfactory abilities. The 'Sniffin' Sticks' set (for a detailed instruction of the Sniffin' Sticks, see Hummel et al., 1997), felt-tip pens filled with phenylethyl alcohol diluted in propylene glycol, was used to investigate olfactory function of the participants using a 'non-biological' odor. The set contained 16 concentrations of phenylethyl alcohol dissolved in logarithmic steps with a stimulus range from 16.3 μM (dilution 16) to 0.54 M (dilution 1).

Androstadienone was obtained, dissolved and handled the same way as described in experiment 1. The exception was that the solution was dissolved into 16 concentrations with a 2-fold increase of liquid concentration, ranging from 0.091 μM (dilution 16) to 3000 μM (dilution 1), in order to match the concentration steps used in the Sniffin' Sticks set and to be sure to cover the whole sensitivity range of all individuals. Two identical sets were alternatively used also in this experiment.

Procedure

Each session was divided into two blocks for threshold measurements for androstadienone or phenylethyl alcohol, randomized with respect to starting order.

Participants were blindfolded with a sleeping mask in order to prevent any visual discrimination of the odor probes. They were presented with triplets of odorants where each individual odor probe was presented, in randomized order, for ~3 s with at least 30 s between the triplets. Only one of the probes in each triplet contained phenylethyl alcohol in different concentrations and the other two contained only the solvent, propylene glycol. Using a forced-choice paradigm, participants had to find out which one of the three probes smelled differently. The triplets were presented only once. Pens were presented in ascending concentrations until the participant had correctly discerned the odorant in two successive trials which then triggered a reversal of the staircase. The test ended after the seventh reversal of the staircase and took on average 30 min (Ehrenstein and Ehrenstein, 1999).

Regarding threshold measurements for androstadienone, we used the same presentation technique as described in experiment 1 with the important exception that this time we used the same ascending staircase paradigm as describe above for the Sniffin' Sticks. Other than in experiment 1, participants did not make any confidence judgments. At the end of the experiment, they rated their hedonic perception of both phenylethyl alcohol and androstadienone on a 10 cm visual analog scale (VAS) ranging from 'extremely unpleasant odor' to 'extremely pleasant odor'.

Data treatment

The mean of the last four staircase reversal points was used as threshold estimate for both androstadienone and phenylethyl alcohol. The differences between groups were assessed using Student's t-test. Analyses of variance (ANOVA) were used to statistically test for differences between groups and interaction effects. Pearson correlation statistics were used in all correlation analyses presented.

A statistical test for bimodality, the DIP intensity test (Giacomelli et al., 1971), was used in order to evaluate the significance of the possibly bimodal sensitivity distribution of androstadienone. The DIP intensity test is a measure of the mean spacing between points in the gap divided by the dispersion of the data. The test does not depend on prior classification of cells and is a highly conservative measure of
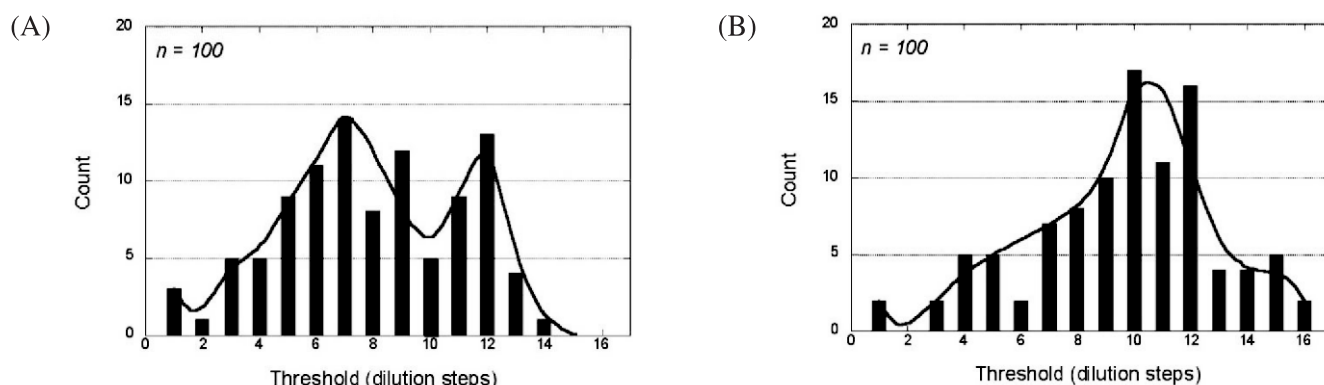
Figure 2 **(A)** Sensitivity distribution for androstadienone expressed in counts per dilution step. The solid line is a smoothed function. Note the anti-mode at dilution step 10. **(B)** Sensitivity distribution for phenylethyl alcohol expressed in count per dilution step. The solid line is a smoothed function.

bimodality since the underlying assumption is a uniform distribution and not a gaussian distribution.

Results and discussion

Mean thresholds for androstadienone and phenylethyl alcohol, expressed in dilution steps, were 9.4 (SEM ± 0.32) and 7.8 (SEM ± 0.30), respectively. The sensitivity distributions of the two odorants are illustrated in Figure 2A,B. Visual inspection suggests that the sensitivity distribution for androstadienone, but not for phenylethyl alcohol, is bimodal.

Two different methods of determining bimodality are currently often used in the literature. One is primarily graphical and relies upon the existence of two modes in the distribution with a clearly visible antimode between these modes. The other definition is based on measures of variance and states that observations that lie two standard deviations from the mean are outliers and belong to another distribution—hence indicating a bimodal distribution. We see reliability problems with both of these methods. Interpretations using graphical methods are dependent on subjective decision criteria of bin sizes. Different bin sizes give different interpretations of the results at hand. Using the method of outliers is dependent on the relative size of the outlier group. In order to assess statistically whether the sensitivity distribution of androstadienone in this experiment is bimodal we used the DIP intensity test (Giacomelli *et al.*, 1971). Since the test is unduly conservative if there are three modes or more, we excluded the three participants that were insensitive to androstadienone (no detection of the highest concentration) from the statistical analyses of bimodality. The DIP intensity test shows that the sensitivity distribution of androstadienone in experiment 2 is bimodally distributed (DIP = 2.07, $P < 0.05$).

The correlation between individual thresholds for androstadienone and phenylethyl alcohol was close to zero [$r(100) = 0.03$, n.s.]. This indicates that the bimodality seen for androstadienone cannot be explained by a general bimodality in olfactory acuity in the sample. This is also evident in the lack of bimodality for phenylethyl alcohol. It should be noted that correlations for individual thresholds between odorants are generally much higher. For example, Cain and Gent (1991) measured thresholds for four odorants; correlations ranged from 0.66 to 0.86 for all six combinations.

Androstadienone and phenylethyl alcohol were perceived differently in hedonic tones by the participants. In general, androstadienone was perceived as unpleasant (mean rating, 4; SD ± 1.91) and phenylethyl alcohol was perceived as pleasant (mean rating, 6.8; SD ± 2.05). The correlation between hedonic rating and individual threshold was low for androstadienone [$r(100) = -0.07$, n.s.], but slightly higher for phenylethyl alcohol [$r(100) = 0.20$, $P = 0.05$]. It could be noted that a significant correlation between olfactory threshold and hedonic ratings has been observed for the related compound androstenone (Dorries *et al.*, 1989; Wysocki and Beauchamp, 1991).

Several studies report that women tend to have a higher olfactory sensitivity than males (Doty, 1986). Here, there was a tendency for women to have a higher sensitivity to androstadienone than men [mean threshold women, 8.28, SEM ± 0.42; men, 7.17, SEM ± 0.43; $t(98) = 1.79$, $P = 0.08$], but not for phenylethyl alcohol [mean threshold women, 9.17, SEM ± 0.46; men, 9.84, SEM ± 0.45, $t(98) = 1.02$, n.s.]. The interaction between substance and sex was, however, significant [$F(1,98) = 4.12$, $P = 0.04$].

To summarize: experiment 2 shows that the sensitivity distribution is bimodal for androstadienone, but not for phenylethyl alcohol. This bimodality cannot be explained by a subgroup of subjects with a general high olfactory sensitivity since there is no correlation between individual thresholds. Accordingly, the supersmellers do not posses a generally high olfactory sensitivity, as judged by phenylethyl alcohol odor thresholds, but rather exhibit a specific sensitivity towards androstadienone.

Experiment 3

As presented above, other odors have previously been reported to have a bimodal sensitivity distribution with one mode that consists of more sensitive individuals. What these bimodal odors all have in common is that they have a high trigeminal factor (Doty *et al.*, 1978). The possibility therefore arises that the demonstrated bimodality in the literature for these odors, as well as the bimodality for androstadienone in this study, are due to the fact that some people have a more sensitive trigeminal system than others (Hummel *et al.*, 2003). The trigeminal system in man is an integrated part of our olfactory perception and mediates sensations such as burning, stinging, warmth, coolness or itching (Hummel, 2000). It is comprised of two major fiber systems, unmyelinated C-fibers and myelinated A_{delta}-fibers (Anton and Peppel, 1991) where C-fibers are involved in the mediation of dull, burning sensations and A_{delta}-fibers mediate sharp and stinging sensations (Torebjork and Hallin, 1974).

When purely olfactory stimuli are presented to one nostril, while the other nostril receives an equal amount of non-odorized air, it is impossible to make correct lateralization judgments (Kobal *et al.*, 1989). Correct lateralization of intranasal stimuli is believed to be due to trigeminal chemoreception based on the fact that neither feedback nor training enhances performance above chance level (Radil and Wysocki, 1998; Wysocki *et al.*, 2003). This disparity between the olfactory and trigeminal system makes it possible to use lateralization judgments as a means to assess the trigeminal impact of an odor (Hummel *et al.*, 2003). In experiment 3, we therefore sought to investigate the possibility that the demonstrated bimodality of androstadienone is due to a subgroup of individuals with high trigeminal sensitivity that reacts to a potential trigeminal, and not olfactory, aspect of androstadienone.

Method

Participants

We recruited 20 participants from those that had participated in experiment 2. Ten of these belonged to the sensitive group in the distribution. That is, they had threshold values for androstadienone in the interval of 10–12 dilution steps (mean threshold 11.3; mean age 23 years). Further, we recruited 10 participants who belonged to the normal group having a threshold value for androstadienone in the interval of 4–8 dilution steps (mean threshold 6.5; mean age 24.6 years). The two groups were balanced for sex and all participants were rewarded with a cinema voucher.

Materials and compounds

Two identical sets, each containing two plastic squeeze bottles, as described above with the exception of a longer spout, were prepared. Each set consisted of one bottle with 30 ml of 3000 µM androstadienone, dissolved in propylene glycol (purity ≥99%), and one with 30 ml propylene glycol. The stimuli were presented to the participants via a hand-held device that ensured an equal airflow of ~15 ml air into each nostril (device and method is described in Hummel *et al.*, 2003).

Procedure

The spouts of the two bottles were placed in the nostrils. Androstadienone was presented to one nostril and pure propylene glycol to the contralateral nostril in a randomized order. The experimenter pressed the hand-held squeezing device containing the two bottles which rendered a puff of air into each nostril at the same time. Participants held onto the spouts to prevent movements that might interfere with their ability to correctly localize the odor by producing mechanical irritation. Forty stimuli (20 targets to each nostril) were presented at an interstimulus interval of ~30 s in order to allow the trigeminal system to recover (Hummel and Kobal, 1998). After each stimulus presentation participants were asked to identify the nostril where the odorant had been presented and also to rate their confidence as described in experiment 1. Stimulus sets were changed in the middle of the session to an unused set in order to maximum the possibility for correct lateralization judgments. Each session required ~30 min of testing.

Data treatment

The difference between means based on the sum of correct lateralization judgments was assessed using Student's *t*-tests.

Results and discussion

We found no evidence of a trigeminal sensation of androstadienone at 3000 µM. The mean correct lateralization score was 20.3 (SEM ± 0.79) for the whole group, which was not significantly different from chance level (i.e. 20) [$t(19) = 0.39$, n.s.]. Participants were well calibrated in respect of confidence judgments of their performance ($O/U = -0.01$). Furthermore, there was no significant difference between groups of different androstadienone sensitivity. The supersmellers had a mean correct lateralization score of 19.4 (SEM ± 0.88) and the normal group 21.2 [SEM ± 1.23, $t(18) = 1.18$, n.s.]. The absence of trigeminal detection indicates that the bimodality in sensitivity to androstadienone is not due to a subgroup of participants with an extra sensitive trigeminal system that reacts to a trigeminal component of androstadienone rather than an olfactory one.

General discussion

The current study investigated individual differences in absolute sensitivity to the putative human pheromone androstadienone. Using the method of constant stimuli, the absolute threshold for androstadienone was determined to be 211 µM (concentration in solution). Several studies have investigated the effects of androstadienone exposure on mood and psychophysiology using 250 µM with or without an odor mask (Grosser *et al.*, 2000; Jacob and McClintock, 2000; Jacob *et al.*, 2001a,b, 2002; Lundstrom *et al.*, 2003). In a recent study (Lundstrom *et al.*, 2003), supersmellers were

able to detect the odor of androstadienone despite a 1% eugenol odor mask. Since some individuals seem to have a specific sensitivity to androstadienone, particular attention must be paid when adjusting the concentration levels when behavioral or physiological effects of non-conscious/non-discriminable exposure of androstadienone are investigated. Although detection of androstadienone could not be tied to the observed effects on mood in the mentioned study, we still maintain that a proper odor discrimination test between test and control odors should be used when measuring exposure effects of putative pheromones.

Several odors have been proven to have a bimodal sensitivity distribution. Only a few of these (see above) are bimodally distributed such that both groups exhibit different sensitivity. For the other bimodal odors (for a review, see Amoore, 1977), bimodality depends on a group of individuals with specific anosmia to the particular odorant, although the choice of stimulus concentration range is of course crucial for this conclusion. From experiment 3 of the current study, it is clear that the observed bimodality is not due to variations in trigeminal sensitivity and therefore most likely of an olfactory origin. Whether this specific sensitivity to androstadienone is due to exposure-induced changes at the receptor level or to genetic factors, as has been suggested for androstenone (Wysocki and Beauchamp, 1984; Yee and Wysocki, 2001), remains to be determined.

Although not significant, the overall results regarding sex differences in olfactory sensitivity to androstadienone in both experiments 1 and 2 point in the direction that women are nominally more sensitive to this putative pheromone than are men. Sex differences in olfaction are largely restricted to tasks that require higher cognitive processing such as odor identification and memory (Doty *et al.*, 1984; Oberg *et al.*, 2002). However, Koelega and Köster (1974) also found that women were in fact more sensitive to androstadienone and other odorants of biological origin than were male participants. However, the sex difference was not present in pre-pubertal children. Dalton *et al.* (2002) showed that an increased sensitivity due to repeated testing was observed among females of reproductive age but not in men. They hypothesized that sex-specific changes in sensitivity might be more likely to occur with biologically relevant odorants. Androstenone, for example, occurs at a higher concentration in male axillae and women are more likely to be normosmic to and to have lower thresholds for androstenone than men which might possible signify an induction of sensitivity in some women who are regularly exposed to male axillary odors. This supposed induction of sensitivity might also be the case for androstadienone. We believe that future studies on the nature of androstadienone sensitivity should focus on the effects of hormonal status and tests of induced sensitivity.

The main conclusion from the experiments presented above is that a subgroup within the population have a specific sensitivity to androstadienone. This is not due to a higher general olfactory acuity or higher trigeminal sensitivity.

Acknowledgements

References

Amoore, **J.E.** (1977) *Specific anosmia and the concept of primary odors.* Chem. Senses Flavor, 2, 267–281.

Anton, **F.** and **Peppel, P.** (1991) *Central projections of trigeminal primary afferents innervating the nasal mucosa: a horseradish peroxidase study in the rat.* Neuroscience, 41, 617–628.

Baron, **R.A.** (1988) *Perfume as a tactic of impression managements in social and organizational settings.* In Toller, S.V. and Dodd, G.H. (eds), Perfumery: The Psychology and Biology of Fragrance. Chapman and Hall, London, pp. 91–104.

Benton, **D.** (1982) *The influence of androstenol—a putative human pheromone—on mood throughout the menstrual cycle.* Biol. Psychol., 15, 249–256.

Benton, **D.** and **Wastell, V.** (1986) *Effects of androstenol on human sexual arousal.* Biol. Psychol., 22, 141–147.

Bjorkman, **M.**, **Juslin, P.** and **Winman, A.** (1993) *Realism of confidence in sensory discrimination: the underconfidence phenomenon.* Percept. Psychophys., 54, 75–81.

Brooksbank, **B.W.**, **Wilson, D.A.** and **MacSweeney, D.A.** (1972) *Fate of androsta-4,16-dien-3-one and the origin of 3-hydroxy-5-androst-16-ene in man.* J. Endocrinol., 52, 239–251.

Cain, **W.S.** and **Gent, J.F.** (1991) *Olfactory sensitivity: reliability, generality, and association with aging.* J. Exp. Psychol. Hum. Percept. Perform., 17, 382–391.

Cutler, **W.B.**, **Friedmann, E.** and **McCoy, N.L.** (1998) *Pheromonal influences on sociosexual behavior in men.* Arch. Sex. Behav., 27, 1–13.

Dalton, **P.**, **Doolittle, N.** and **Breslin, P.A.** (2002) *Gender-specific induction of enhanced sensitivity to odors.* Nat. Neurosci., 5, 199–200.

Dorries, **K.M.**, **Schmidt, H.J.**, **Beauchamp, G.K.** and **Wysocki, C.J.** (1989) *Changes in sensitivity to the odor of androstenone during adolescence.* Dev. Psychobiol., 22, 423–435.

Doty, **R.L.** (1986) *Reproductive endocrine influences upon olfactory perception: a current perspective.* J. Chem. Ecol., 12, 497–511.

Doty, **R.L.**, **Brugger, W.P.E., Jurs, P.C., Orndorff, M.A., Snyder, P.J.** and **Lowry, L.D.** (1978) *Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans.* Physiol. Behav., 20, 175–185.

Doty, **R.L.**, **Shaman, P., Applebaum, S.L., Giberson, R., Siksorski, L.** and **Rosenberg, L.** (1984) *Smell identification ability: changes with age.* Science, 226, 1441–1443.

Ehrenstein, **W.H.** and **Ehrenstein, A.** (1999) *Psychophsical methods.* In Windhorst, U. and Johansson, H. (eds), Modern Techniques in Neuroscience Research. Springer Verlag, Berlin, pp. 1211–1241.

Giacomelli, **F.**, **Wiener, J.**, **Kruskal, J.B.**, **Pomeranz, J.V.** and **Loud, A.V.** (1971) *Subpopulations of blood lymphocytes demonstrated by quantitative cytochemistry*. J. Histochem. Cytochem., 19, 426–433.

Gower, **D.B.** and **Ruparelia, B.A.** (1993) *Olfaction in humans with special reference to odorous 16-androstenes: their occurrence, perception and possible social, psychological and sexual impact*. J. Endocrinol., 137, 167–187.

Grosser, **B.I.**, **Monti-Bloch, L.**, **Jennings-White, C.** and **Berliner, D.L.** (2000) *Behavioral and electrophysiological effects of androstadienone, a human pheromone*. Psychoneuroendocrinology, 25, 289–299.

Hummel, **T.** (2000) *Assessment of intranasal trigeminal function*. Int. J. Psychophysiol., 36, 147–155.

Hummel, **T.**, **Futschik, T.**, **Frasnelli, J.** and **Huttenbrink, K.B.** (2003) *Effects of olfactory function, age, and gender on trigeminally mediated sensations: a study based on the lateralization of chemosensory stimuli*. Toxicol. Lett., 140–141, 273–280.

Hummel, **T.** and **Kobal, G.** (1998) *Chemosensory event-related potentials to trigeminal stimuli change in relation to the interval between repetetive stimulation of the nasal mucosa*. Eur. Arch. Otorhinolaryngol., 256, 16–21.

Hummel, **T.**, **Sekinger, B.**, **Wolf, S.R.**, **Pauli, E.** and **Kobal, G.** (1997) *'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold*. Chem. Senses, 22, 39–52.

Jackman, **P.J.** and **Noble, W.C.** (1983) *Normal axillary skin microflora in various populations*. Clin. Exp. Dermatol., 8, 259–268.

Jacob, **S.** and **McClintock, M.K.** (2000) *Psychological state and mood effects of steroidal chemosignals in women and men*. Horm. Behav., 37, 57–78.

Jacob, **S.**, **Hayreh, D.J.** and **McClintock, M.K.** (2001a) *Context-dependent effects of steroid chemosignals on human physiology and mood*. Physiol. Behav., 74, 15–27.

Jacob, **S.**, **Kinnunen, L.H.**, **Metz, J.**, **Cooper, M.** and **McClintock, M.K.** (2001b) *Sustained human chemosignal unconsciously alters brain function*. Neuroreport, 12, 2391–2394.

Jacob, **S.**, **Garcia, S.**, **Hayreh, D.** and **McClintock, M.K.** (2002) *Psychological effects of musky compounds: comparison of androstadienone with androstenol and muscone*. Horm. Behav., 42, 274–283.

Juslin, **P.**, **Olsson, H.** and **Winman, A.** (1998) *The calibration issue: theoretical comments on Suantak, Bolger, and Ferrell (1996)*. Organ. Behav. Hum. Dec., 73, 3–26.

Kobal, **G.**, **Van Toller, S.** and **Hummel, T.** (1989) *Is there directional smelling?* Experientia, 45, 130–132.

Koelega, **H.S.** and **Köster, E.P.** (1974) *Some experiments on sex differences in odor perception*. Ann. N.Y. Acad. Sci., 23, 234–246.

Labows, **J.N.** and **Wysocki, C.J.** (1984) *Individual differences in odor perception*. Perfumer & Flavorist, 9, 21–26.

Lison, **M.**, **Blondheim, S.H.** and **Melmed, R.N.** (1980) *A polymorphism of the ability to smell urinary metabolites of asparagus*. Br. Med. J., 281, 1676–1678.

Lundstrom, **J.N.**, **Goncalves, M.**, **Esteves, F.** and **Olsson, M.J.** (2003) *Psychological effects of subthreshold exposure to the putative human pheromone 4,16-androstadien-3-one*. Horm. Behav. In press.

Mallet, **A.I.**, **Holland, K.T.**, **Rennie, P.J.**, **Watkins, W.J.** and **Gower, D.B.** (1991) *Applications of gas chromatography-mass spectrometry in the study of androgen and odorous 16-androstene metabolism by human axillary bacteria*. J. Chromatogr., 562, 647–658.

Nixon, **A.**, **Mallet, A.I.** and **Gower, D.B.** (1988) *Simultaneous quantification of five odorous steroids (16-androstenes) in the axillary hair of men*. J. Steroid. Biochem., 29, 505–510.

Oberg, **C.**, **Larsson, M.** and **Bäckman, L.** (2002) *Differential sex effects in olfactory functioning: the role of verbal processing*. J. Int. Neuropsychol. Soc., 8, 691–698.

Odeigah, **P.G.** (1994) *Smell acuity for acetone and its relationship to taste ability to phenylthiocarbamide in a Nigerian population*. East Afr. Med. J., 71, 462–466.

Ohloff, **G.**, **Maurer, B.**, **Winter, B.** and **Giersch, W.** (1983) *Structural and configurational dependency of the sensory process in steroids*. Helv. Chim. Acta, 66, 192–217.

Patterson, **R.L.S.** and **Griffiths, N.M.** (1969) Nature, 22, 15.

Pierce, **J.D.**, **Jr**, **Doty, R.L.** and **Amoore, J.E.** (1996) *Analysis of position of trial sequence and type of diluent on the detection threshold for phenyl ethyl alcohol using a single staircase method*. Percept. Mot. Skills., 822, 451–458.

Prelog, **V.**, **Ruzicka, L.** and **Wieland, P.** (1945) *Über ein neues stereoisomeres des oestriols*. Helv. Chim. Acta, 28, 250–256.

Radil, **T.** and **Wysocki, C.J.** (1998) *Spatiotemporal masking in pure olfaction*. Ann. N. Y. Acad. Sci., 855, 641–644.

Rennie, **P.J.**, **Gower, D.B.** and **Holland, K.T.** (1991) *In-vitro and in-vivo studies of human axillary odour and the cutaneous microflora*. Br. J. Dermatol., 124, 596–602.

Savic, **I.**, **Berglund, H.**, **Gulyas, B.** and **Roland, P.** (2001) *Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans*. Neuron, 31, 661–668.

Thorne, **F.**, **Neave, N.**, **Scholey, A.**, **Moss, M.** and **Fink, B.** (2002) *Effects of putative male pheromones on female ratings of male attractiveness: influence of oral contraceptives and the menstrual cycle*. Neuroendocrinol. Lett., 23, 291–297.

Thysen, **B.**, **Elliott, W.H.** and **Katzman, P.A.** (1968) *Identification of estra-1,3,5(10),16-tetraen-3-ol (estratetraenol) from the urine of pregnant women (1)*. Steroids, 11, 73–87.

Torebjork, **H.E.** and **Hallin, R.G.** (1974) *Responses in human A and C fibres to repeated electrical intradermal stimulation*. J. Neurol. Neurosurg. Psychiatry, 37, 653–664.

White, **T.L.** (1998) *Olfactory memory: the long and short of it*. Chem. Senses, 23, 433–441.

Wysocki, **C.J.** and **Beauchamp, G.K.** (1984) *Ability to smell androstenone is genetically determined*. Proc. Natl Acad. Sci. USA, 81, 4899–4902.

Wysocki, **C.J.** and **Beauchamp, G.K.** (1991) *Individual differences in olfaction*. In Wysocki, C.J. and Kare, M.R. (eds), Genetics of Perception and Communications. Dekker, New York, pp. 353–373.

Wysocki, **C.J.**, **Pierce, J.D.** and **Gilbert, A.N.** (1991) *Geographic, cross-cultural, and individual variation in human olfaction*. In Getchell, T.V., Bartoshuk, L.M., Doty, R.L. and Snow, J. (eds), Smell and Taste in Health and Disease. Raven Press, New York, pp. 287–314.

Wysocki, **C.J.**, **Cowart, B.J.** and **Radil, T.** (2003) *Nasal trigeminal chemosensitivity across the adult life span*. Percept. Psychophys., 65, 115–122.

Yee, **K.K.** and **Wysocki, C.J.** (2001) *Odorant exposure increases olfactory sensitivity: olfactory epithelium is implicated*. Physiol. Behav., 72, 705–711.