Changes in the Odor Quality of Androstadienone During Exposure-Induced Sensitization

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Abstract

Androstadienone is a steroid found in human sweat and other secretions. It has been widely proposed as a candidate for a human pheromone. As an odorant it possesses some unique properties. Here we demonstrate that, firstly, there is a very wide range of thresholds in the human population, and they are not normally distributed. Secondly, repetitive exposure causes a decrease in detection threshold of more than four orders of magnitude, and thirdly, accompanying this sensitization process is a change in the perceived odor quality. Those with low to intermediate sensitivities ascribe to it a wide range of odor descriptors across the hedonic scale, but as these individuals become sensitized, their description changes to predominantly putrid. We propose that this change in odor quality reflects the presence of at least two receptor populations for androstadienone; a low-affinity receptor conveying pleasant odor qualities and a high-affinity receptor mediating unpleasant odor qualities. We further propose that repetitive exposure results in the increased expression of the high-affinity receptor thereby shifting the balance of perception to the negative end of the hedonic scale.

Key words: odor, olfaction, olfactory, pheromone, smell

Introduction

Androstadienone is a member of the family of 16 androstenes, steroid compounds that are found in human secretions. It is present in peripheral plasma, and it occurs at a higher concentration in men (0.98 μg/l) than in women (0.36 μg/l) (Brooksbank et al., 1972). It has also been found in axillary secretions, axillary hair, and on the surface of the skin. Androstadienone, when applied directly to the human vomeronasal organ (VNO), was reported to generate potentials in the VNO that were greater in women than in men (Monti-Bloch and Grosser, 1991; Berliner, 1994). Later studies reported that it induced decreases in respiratory frequency, cardiac frequency, and galvanic skin response and caused increases in body temperature, cortical alpha-wave activity, parasympathetic tone (Grosser et al., 2000), and caused a gender specific activation of the hypothalamus in women (Savic et al., 2001). In addition to these physiological changes, it was claimed that picogram quantities of androstadienone applied directly to the human female VNO reduced discomfort and tension (Grosser et al., 2000). However, the existence of a functional vomeronasal system in humans is not generally accepted (see Meredith, 2001, for a review). While the VNO is mainly responsible for pheromone detection in rodents, mammals, such as rabbit and pig, are able to detect pheromones through the main olfactory system (Hudson and Distel, 1986; Dorries et al., 1997), and vomeronasal receptors are expressed at the mRNA level in the human olfactory mucosa (Rodriguez et al., 2000).

In spite of reports that androstadienone produced little or no effect on the olfactory epithelium (Monti-Bloch and Grosser, 1991; Berliner, 1994) it does have an odor. It has been described as a "low-odor androstene" compared to androstenone (Pause, 2004). Reports also describe a "strong urine" (Ohloff et al., 1983), "musky" (Jacob et al., 2002) and "unpleasant" odor (Lundstrom et al., 2003b), and it has been given "pleasantness" ratings of 40/100 on a bipolar visual analog scale (Savic et al., 2001), 4.8 on a 9-point scale (Bensafi et al., 2004a), and +1 on a ±10 analog scale (Gulyas et al., 2004). Because of this apparent conflict—possessing an odor but lacking an effect on the olfactory epithelium—we undertook a study on the olfactory detection threshold and odor quality of androstadienone. In the process, we discovered that the detection of androstadienone was experience dependent. We show that detection thresholds during repetitive exposure decrease and that this sensitization is accompanied by perceptual odor quality changes.

Materials and methods

Odorants

The odorants used were androstadienone (4,16-andro-stadien-3-one) of minimum 98% pure by thin layer chromotography obtained from Steraloids (Cat. No. A0570-000; Newport, RI) and amyl acetate (Sigma Chemical Co., Poole, England), a substance with an apple/banana-like odor. The binary androstadienone dilutions were made from a stock solution of 12.3 mM (0.3% w/v) in silicone oil (Dow Corning 200/350cSt., Midland, MI), and binary dilutions of amyl acetate were made from a 4.28 mM (0.064% v/v) stock solution in the same silicone oil.

Ethical approval

The study conformed to the Declaration of Helsinki.

Ethical approval (#01/4353) was granted by the Bro Taff Health Authority Local Research Ethics Committee.

Subjects

The 53 subjects were from the student population of the university, and none had a history of olfactory dysfunction or respiratory disease. The average age was 26.5 ± 0.95 (±SE; $N = 53$); there were 23 men (average age = 26.2 ± 0.9, range 20–34) and 30 women (average age 26.7 ± 1.6, range 20–44). All subjects gave their informed consent and were paid for their participation.

Threshold test

The single staircase procedure was used (Doty and Laing, 2003). This involved a three-alternative forced choice test of a series of binary dilutions. Nineteen binary dilutions were made by serial dilution from a starting concentration of 0.3% (w/v) androstadienone in silicone oil (12.3 mM) and 0.064% (v/v) amyl acetate in silicone oil, resulting in a final concentration for androstadienone of 2.35×10^{-8} M. Starting from a low concentration, the odors were presented in ascending order in 250-ml glass bottles containing 20 ml of liquid. Each odor concentration was presented along with two blanks of the diluent. When the subject correctly identified the bottle

containing the odor twice, the staircase was reversed. The average of the last four of seven staircase reversal points was taken as the threshold.

Odor descriptors

At the end of the threshold test subjects were asked to sniff 0.3% androstadienone and to select one or more smell descriptors from the (Stevens and O'Connell, 1995) list (Table 1).

Repetitive exposure trial

A group of five men (average age 28.3 ± 1.9) and three women (average age 24.1 ± 2.3) with low to intermediate androstadienone thresholds were selected for the repetitive exposure trial. Their thresholds for both androstadienone and amyl acetate were determined, and they were asked to provide descriptors of the androstadienone from Table 1. They were given a 20-ml bottle containing 2 ml 0.3% androstadienone and were instructed to sniff it for 3 min three times daily. They returned every 2 days over the 2-week exposure period for further threshold tests. At the end of the trial, they were asked to select smell descriptors again, and their amyl acetate thresholds were tested for a second time.

Results

The detection thresholds of 53 subjects (23 men and 30 women) for androstadienone were not normally distributed (Figure 1b). The frequency distribution histogram was best fitted by the sum of four Gaussians, while the frequency distribution of the same subjects for amyl acetate thresholds was fitted by a single Gaussian (median = 3.9×10^{-6} M; dashed line, Figure 1a). Subjects were asked to give one or more descriptors (see Table 1) for the quality of the androstadienone. In Figure 2 the thresholds of the subjects are plotted as a function of their choice of the six major categories of smell quality; putrid, vegetable, floral, woody, minty, and fruity as given in Table 1. The frequency and median detection threshold of each odor group are included in the legend to Figure 2. The 53 subjects gave 78 descriptors. The "putrid" descriptor was the most common, chosen by 39 subjects (50%). It included the greatest range of thresholds and was associated with

Table 1 Smell descriptors

| Putrid	Vegetable	Floral	Woody	Minty	Fruity
Sweaty	Green pepper	Rose	Pine	Wintergreen	Lemon
Urine	Cucumber	Lavender	Cedar	Peppermint	Orange
Rancid	Celery	Violet	Sandalwood	Camphor	Strawberry
Sour	Green bean	Lilac	Hickory	Eucalyptus	Cherry
Faecal	Cabbage	Muguet	Balsam	Menthol	Melon
Other, please specify	Other, please specify	Other, please specify	Other, please specify	Other, please specify	Other, please specify

Taken from Stevens and O'Connell (1995).

(a) Amyl acetate

(b) Androstadienone
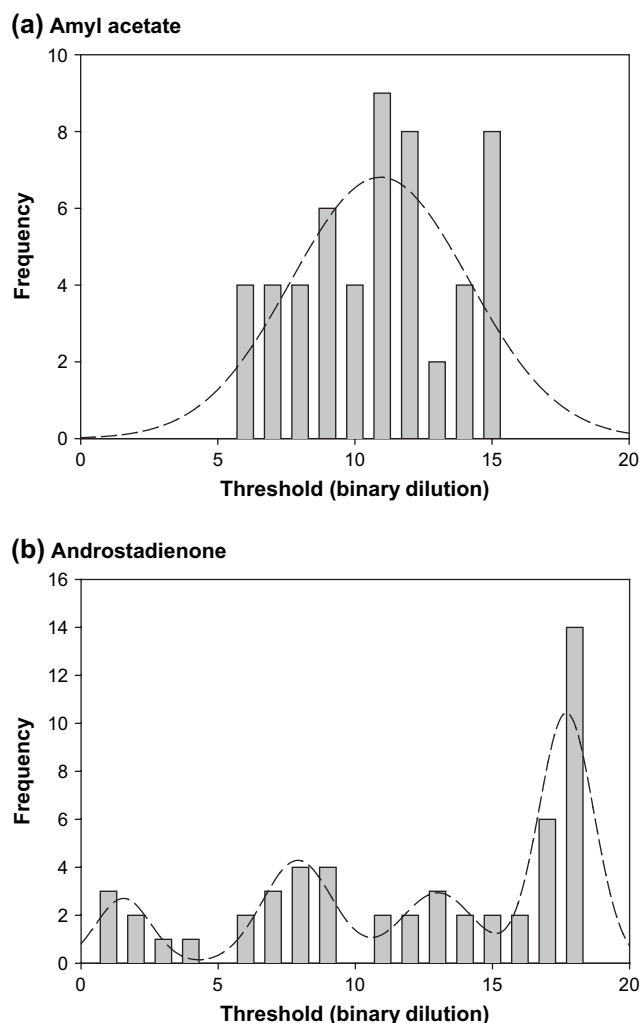
Figure 1 **(a)** The frequency distribution of amyl acetate thresholds. The thresholds are presented as binary dilutions of a stock solution of 0.064% (v/v) of amyl acetate in silicone oil. The dashed line represents the best fit of a single Gaussian with a median value of 3.9×10^{-6} M and correlation coefficient $R = 0.942$; $N = 53$. The Gaussians were fitted using the Marquardt–Levenberg algorithm which iteratively minimizes the sum of the squares of the residuals until convergence. **(b)** The frequency distribution of androstadienone thresholds. The threshold is presented as binary dilution of a stock solution of 0.3% (w/v) of androstadienone in silicone oil. The dashed line is the best fit of the sum of four Gaussians with median values of 4.8×10^{-3} M, 6.9×10^{-5} M, 2.26×10^{-6} M, and 6.6×10^{-9} M, correlation coefficient $R = 0.989$; $N = 53$.

the highest sensitivity (median threshold = 5.3×10^{-6} M). Fewer people chose "minty" and "fruity" descriptors (five and six people, respectively). The minty descriptor was chosen by the least sensitive individuals (median threshold = 3.2×10^{-5} M). The "vegetable" descriptor was the second most common choice (14 subjects, 18%) and tended to be selected by those with an intermediate sensitivity (median threshold = 5.7×10^{-6} M).

Not only was the threshold distribution of androstadienone anomalous but also it was noticed that thresholds tended to decrease on subsequent tests. A repetitive exposure
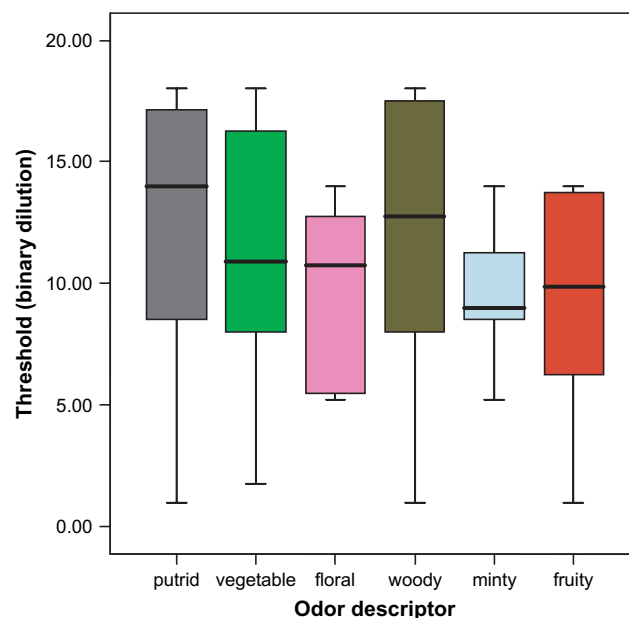
Figure 2 Box plot of androstadienone thresholds as a function of smell descriptor. The categories of odor descriptor are taken from Stevens and O'Connell (1995). The boxes extend from the 25th to the 75th percentile, and the median is represented as a bar with the whiskers extending from the smallest to the largest observed values. Fifty-three subjects chose a total of 78 descriptors as follows: 39 putrid (50%), 14 vegetable (18%), 5 floral (6.4%), 9 woody (11.5%), 5 minty (6.4%) and 6 fruity (7.7%). The median values for each descriptor were as follows: putrid = 5.3×10^{-6} M (12.2 dilution), vegetable = 5.7×10^{-6} M (11.1 dilution), floral = 3.1×10^{-5} M (9.65 dilution), woody = 1.0×10^{-5} M (11.3 dilution), minty = 3.2×10^{-5} M (9.6 dilution), fruity = 4.5×10^{-5} M (9.1 dilution).

trial was performed in which subjects with low to intermediate sensitivities were selected and required to sniff androstadienone for 3 min three times daily for 2 weeks. Their initial thresholds for androstadienone were 5.56 ± 1.28 mM (mean ± SE), corresponding to binary dilution 4.4 ± 1.9 for the men and 0.12 ± 0.02 mM (binary dilution 7.8 ± 0.2) for the women. Following repetitive exposure, these fell to 0.23 ± 0.03 μM (binary dilution 17.0 ± 0.4) for the men and to 0.30 ± 0.58 μM (binary dilution 17.3 ± 0.4) for the women. Since there was no significant difference between the results for men and women (Kruskall–Wallis test, observed significance 0.174, 0.763, 0.245, 0.164, 1.0, 0.24, 0.639, 0.180 for men vs. women at test sessions 1–8, respectively), the data were pooled and are presented in Figure 3. A nonparametric one-way repeated measures analysis of variance (Friedman test) demonstrated that there was a significant effect of exposure training (χ^2 value = 25.833, 7 degrees of freedom, $P < 0.001$) on androstadienone threshold.

Thresholds for amyl acetate, measured before and after sniff training, did not change. They were (mean binary dilution ± SE) 10.6 ± 0.9 and 12.6 ± 1.9 before and 10.3 ± 1.2 and 13.1 ± 1.4 after, for men and women, respectively (Wilcoxon signed rank test, $P = 1.000$).

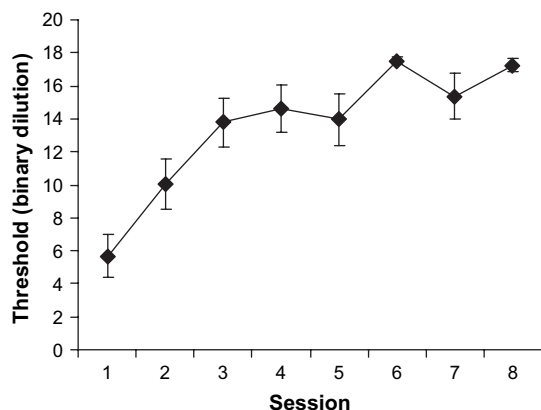### Smell descriptors before and after repetitive exposure trial

The group selected for the repetitive exposure trial were required to select the best descriptor (Table 1) to match their perception of androstadienone before the 2-week exposure program began and again at the end. Initially, a wide range of descriptors were used as used for the larger group for the first part of this study—putrid, vegetable, floral, woody, minty, and fruity (Figure 4a). However, following exposure, the androstadienone thresholds of all eight participants fell (see results above and Figures 3 and 4), and their range of descriptors diminished—focussing on the putrid, vegetable,

Figure 3 Exposure-dependent sensitization to androstadienone. The *x*-axis represents successive threshold determinations over a 2-week period. Subjects sniffed 0.3% androstadienone for 3 min, three times daily. The threshold is presented as binary dilution of a stock solution of 0.3% (w/v) of androstadienone in silicone oil. Data represent the mean ± SE (bars) from eight subjects (five men, three women). There was a significant effect of exposure on threshold (see Results for details).

and woody qualities. The floral, minty, and fruity qualities were absent. The frequency of the putrid descriptor rose from 29% before the repetitive exposure trial to 58% following the trial.
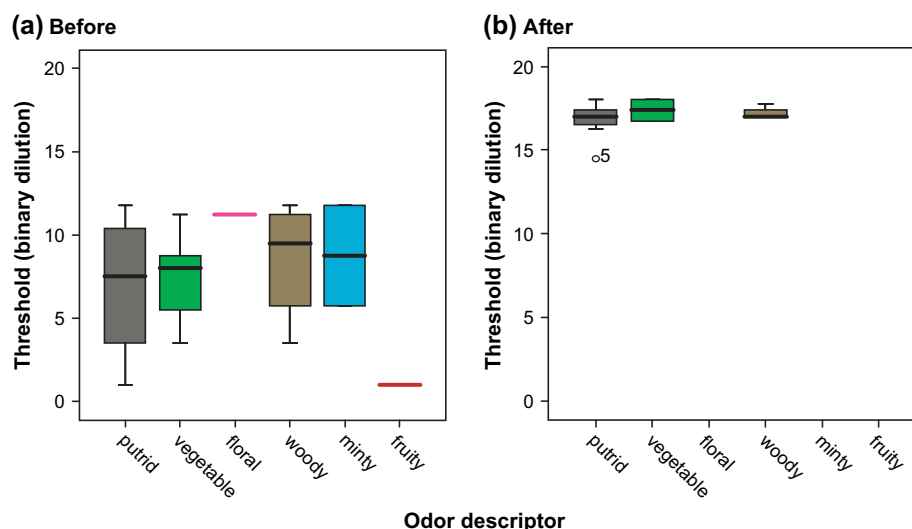
Discussion

Men and women have a wide range of sensitivities to androstadienone in common with the related steroid, the pig pheromone androstenone (Labows and Wysocki, 1984). Lundstrom *et al.* (2003b) suggested that the sensitivity distribution for androstadienone was bimodal but reported a single absolute detection threshold of 221 μM. In this present study we found that some people can smell androstadienone at below 1 μM, while others cannot detect it at all. The threshold distribution was multimodal. Taking into account the experience-dependent changes in threshold (see Figure 3) it can be predicted that, in any given population, the threshold for androstadienone and other related steroids found in human secretions is in a dynamic state dependent upon the exposure history of the individuals in that population. The important conclusion from this is that the threshold distribution of any population studied will be distributed over a wide range and will not exhibit the more usual normal distribution characteristic of most other odors.

Those with higher sensitivities to androstadienone describe it as having a putrid odor—a description that includes "sweaty," "urinous," etc (see Table 1), while for those with intermediate sensitivities, there was a wider range of descriptors including vegetable, floral, minty, and fruity. The differences of concentrations used in earlier studies would explain the contradictory odor qualities ascribed to androstadienone.

Figure 4 Box plot of androstadienone thresholds as a function of smell descriptor taken **(a)** before and **(b)** after exposure-dependent sensitization. Subjects (five men, three women) were sensitized by sniffing 0.3% androstadienone for 3 min, three time daily. As thresholds decreased, odor descriptors became more limited, dominated by the putrid descriptor. Before exposure-dependent sensitization, eight subjects chose 17 descriptors as follows; five putrid (29%), three vegetable (18%), one floral (6%), five woody (29%), two minty (12%), one fruity (6%). Following exposure-dependent sensitization, the eight subjects chose 12 descriptors as follows; seven putrid (58%), two vegetable (17%), zero floral (0%), three woody (25%), zero minty (0%), and zero fruity (0%).

In addition, repetitive exposure to androstadienone caused a reduction in threshold in both men and women of more than four orders of magnitude from 3.5×10^{-3} M to 0.3×10^{-6} M (Figure 3). No such reduction in threshold was observed for amyl acetate under the same conditions (Wang *et al.*, 2004). These properties, in particular the large threshold range spanning four orders of magnitude, may go some way to explain the variable and sometimes contradictory results; for example, different effects on mood have been reported for androstadienone, from no effect (Bensafi *et al.*, 2003) to a non-gender specific increase in positive stimulated mood (Jacob and McClintock, 2000) which was dependent upon the gender of the experimenter (Jacob *et al.*, 2001) or only occurred in specific contexts (Bensafi *et al.*, 2004a). While some subjects may have had a very low threshold and were therefore very sensitive to a given concentration of the androstene, others, with a high threshold, may have been insensitive.

The exposure-dependent gain in androstadienone sensitivity we found was accompanied by a change in perceived odor quality. Our selected low-sensitivity subjects described androstadienone as having a wide range of odor qualities pre-exposure; putrid, vegetable, woody, floral, minty, and fruity. Following exposure-induced sensitization, the only descriptors were putrid, vegetable, and woody independent of the androstadienone concentration. The qualities of floral, minty, and fruity were lost during the sensitization process. Applying Occam's razor, this result could support the hypothesis that there are at least two odor channels; a broadly pleasant channel with floral, minty, and fruity qualities and an unpleasant channel with putrid qualities. These channels could be represented by receptors, one with a high-affinity—the putrid receptor—and the other a low-affinity receptor associated with more diffuse range of more pleasant odor qualities (Polak, 1973; Stevens and O'Connell, 1995). As sensitivity increases, the high-affinity receptor is activated at androstadienone levels well below those necessary to activate the low-affinity receptor, and the dominant odor quality becomes putrid.

Androstadienone has been widely hailed as the leading candidate for a human pheromone or chemosignal (Berliner, 1994; Grosser *et al.*, 2000; Jacob and McClintock, 2000; Jacob *et al.*, 2001; Savic *et al.*, 2001; Jacob *et al.*, 2002; Bensafi *et al.*, 2003; Lundstrom *et al.*, 2003a,b; Bensafi *et al.*, 2004a,b; Cornwell *et al.*, 2004; Gulyas *et al.*, 2004). We have demonstrated that it possesses some unique properties as an odor. This study raises two questions; firstly, what is the functional significance of this sensitization process? And secondly, why should an odor quality change accompany this sensitization process? Similar increases in sensitivity in human subjects, both men and women, have been observed with androstenone (Wysocki *et al.*, 1989; Wang *et al.*, 2004), and this has been related to an increase in the number of peripheral receptor cells or the number of receptors expressed per cell (Wang *et al.*, 2004). Increases in sensitivity of this nature are rare, and usually the reverse pro-cess, habituation or adaptation, occurs following repetitive exposure. Exposure-dependent increases in sensitivity have been observed, in women of reproductive age, to citravla and benzaldehyde, but such changes were not observed in men (Dalton *et al.*, 2002), suggesting that this may be a different phenomenon. There are few odorants whose odor quality changes, and usually the change is related to intensity, becoming more unpleasant with increasing intensity. The reverse is true for androstadienone; the unpleasant odor quality develops when the sensitivity increases and is in response to very low concentrations.

Future studies need to address the issue of what makes this small class of odorants, sex steroid-like compounds, different to the vast majority of smells. These steroids are relatively nonvolatile and would only be detected during close encounters. Repeated exposure would result in a learning process that would enhance any response they may induce. Such properties would suit a chemical signaling compound. In human interactions, there may be a function for such compounds, limited to key life stages and between closely connected individuals, to communicate information relevant only to those individuals. This might occur, for example, between mothers and infants and between sexual partners.

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