5-α-androst-16en-3α-on: A Male Pheromone? A Brief Report

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Olfactory cues play a prominent, yet underestimated role in shaping emotional attitudes towards conspecifics. Among humans olfactory cues can have effects on behavior. In a rating study (n = 290) females rated the smell of androstenone. The emotional reaction to androstenone changed during the menstrual cycle. Females rated the main component of male body odor unattractive. This changed to a neutral emotional response at the conceptive optimum around ovulation. The finding has direct consequences for hypotheses concerning the evolutionary loss of estrus. It is suggested that the cyclic-dependent emotional rating of androstenone may facilitate active female choice of sex partners and may be a proximate cue for female mate-choice.

KEY WORDS: Androstenol; Androstenone; Smell; Menstrual cycle; Sexual strategies.

INTRODUCTION

Olfactory cues play a prominent role in animal sexual behavior. In humans such cues are difficult to isolate and related discussions have been quite controversial (Doty 1976). Nonetheless, humans are capable of discriminating the sexes by olfactory cues alone (Hold and Schleidt 1977). Sex differences in the composition of human axillary sweat may be the basis of such discrimination, where one candidate is the dimorphism in axillary gland production of 5-α-androst-16en-3α-ol and 5-α-androst-16en-3α-on which is higher in males (Brooksbank et al., 1974).

Many authors have speculated that both androstenone and androstenol are male pheromones. These speculations lead to the question of whether and how females might perceive them. Filsinger et al. (1985) showed that the application of androstenone to females led to negative descriptions of males whereas the application of androstenol resulted in a description of males as being sexually attractive. Cowley et al. (1977) showed that females wearing androstenol-prepared masks judged males more positively than con-

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trols. Kirk-Smith et al. (1978) examined the influence of boar pheromone (androstenol) on subjects' judgments of photographs. Male and female subjects rated photographed women as "sexier," "more attractive," and "better" in the presence of androstenol. Thus it has repeatedly been shown that females find this smell attractive and that the perception of this odor can increase female sexual arousal (McCollough et al. 1981). These results then indicate that androstenol can induce positive and androstenone negative attitudes towards males, suggesting that androstenol may be a male sex pheromone, enhancing attractiveness.

On the other hand, some results dispute this "androstenol-male pheromone" hypothesis. For instance, a direct analysis of fresh human sweat does not always indicate that both substances are present. Neither substance can be found persistently in fresh sweat in concentrations above the olfactory threshold. With regard to this, Labows et al. (1979) concluded that both 5-α-androst-16en-3α-ol with its sandalwood-like smell and 5-α-androst-16en-3α-on with a urine-like smell were produced by the action of enzymes and bacteria. In contrast, Amoore et al. (1977) postulated that the primary product could be 5-α-androst-16en-3α-ol which under exposure to oxygen becomes 5-α-androst-16en-3α-on.

If androstenol promotes female sexual attraction towards males it should have a role in the hypothesized sexual signaling system. Problems arise, however, with determining the function of androstenone, which induces negative female emotions towards males. In fact, androstenone is the more prominent odor. As males are not always sweating, the odor of androstenone could prevail, whereas the fresh sweat odor of androstenol might disappear quickly.

The situation is complicated further by the fact that olfaction in females is modulated by the menstrual cycle (Doty 1976). Sensitivity to smells appears to peak at ovulation. Doty et al. (1981) showed a direct correlation between estrogen, luteinizing hormone, and heightened olfactory sensitivity. These changes in olfaction during menstrual cycle also apply to androstenol. Benton (1982) showed that the application of androstenol to the upper lip of females made them rate their mood at the time of ovulation as more submissive. In contrast, a study by Filsinger and Monte (1986) found no clear link between sexual history and the perception of androstenone. This negative result might have been due to a research design that did not discriminate between females who take hormonal contraceptives and those who do not.

These mixed findings do not rule out the possibility that female hormonal status may directly influence the perception of androstenone and androstenol. Overall, this suggests the existence of two different olfactory signals: androstenol which induces female attraction to males; and androstenone which induces negative attitudes in females.

One "unconscious" mechanism associated with these menstrual changes might be that of olfactory perceptions. Ovulation in humans cannot be perceived consciously by either males or females. Changes here could

be important in menstrual timing with regard to conspecifics and the perception of potential partners' olfactory cues. The latter phenomenon would be supported if the perception of androstenone varied with cycle and if such variation induced changes in mood tending to raise or lower frequency of approaches to males, and thus frequencies of copulations with specific males.

METHOD

For the experiment 5 mg 5-α-androst-16en-3α-one (Sigma A-8008) was dissolved in 5 ml absolute ethanol (Cowley et al. 1977), and 0.1 ml of the solution were administered on Dragoco-smelling-pads and dried at room temperature.

The design was cross-sectional and so not subject to order effects, as might be the case if females were followed-up through a complete cycle.

The participants consisted of 289 female students (mean age = 23.26; S.D. = 5.90). The test-sheets were handed out to them together with a questionnaire eliciting basic data (age, sex, profession, use of contraceptives, living circumstances) and the day of menstrual cycle counted from the first day of menstrual flow.

Menses might differ in duration and thus it is not possible to determine the exact date of ovulation. Variation in time of female ovulation would actually work against our hypotheses. On this basis, a positive result is arguably more significant than for a study using more accurate estimates of date of ovulation, as would be obtained using a temperature measuring method (Döring 1990). Another possible source of inaccuracy in the method of estimating time of ovulation is variation in cycle length. Accordingly, all females were excluded from the study who indicated a cycle length longer than 28 days. Thus, this study has clear but not unsurmountable methodological drawbacks.

A free evaluation of the test odor and an odor profile consisting of 18 pairs of adjectives (c.f. Benton 1982; Kirk-Smith et al. 1978) were administered. The standardized profile was used to conceal the two adjective pairs, attractive-unattractive, and pleasant-unpleasant, which describe the hedonic aspects under interest. The sequence of the adjective pairs was randomized to suppress order effects. Subjects were then asked to rate the odor on a seven-point rating scale. In pursuit of the test hypothesis, only the two pairs describing the hedonic dimension of perception were analyzed in a combined scale.

Experimental controls posed a basic problem in this study, because overall sensitivity to odors could change with menstrual cycle and different odors might be perceived the same way. Administration of rose-water or absolute ethanol was not a possible control solution. It seemed easier to compare subjects who were ovulating with those who were not. The task of categorization was straightforward since women who take hormonal contraceptives do not ovulate.

RESULTS

36.3% (105) of the females were using hormonal contraceptives and 63.7% (N = 184) did not. As might have been expected, a higher percentage of those living together with a male took contraceptives (50.5%) compared with those who lived alone or with their parents (30.3%). Not all females can smell androstenone. In our experiment 92.9% (n = 171) of the females who did not take the pill detected it. A comparable percentage was found among those females who took the pill (n = 96, 91.4%). Thus taking the pill has no significant effect on being anosmic to androstenone.

The two scales of pleasantness and attractiveness correlated highly (rank spearman r = 0.61 and 0.85 for pill-takers and non-pill-takers). Thus it seemed reasonable to combine the data from the two questions to a single hedonic scale to reduce the number of statistical tests necessary. In the combined scale a hedonic rating would be maximally 14 and minimally 2. The resulting distribution was not uniform, for either females using the pill (Kolgoromov-Smirnov Z = 2.9, $p < 0.0001$) or for females who did not (Kolgoromov-Smirnov Z = 3.08, $p < 0.0001$). Both data sets showed considerable skewness (females who take the pill: 0.65; females who do not take the pill: 0.21). The majority of females, independent of contraceptives, perceive androstenone as negative, the mean score on the scale being 6.38 (sd 3.02).

The analysis of the hedonic part of the rating scale showed differences between females taking no contraceptives and females taking the pill. Females on oral contraceptives rate androstenone less pleasant/attractive (mean 6.00, sd 2.96) then females not taking the pill (mean 6.67, sd 2.89) (Kruskal-Wallis-One-Way Anova, $\chi^2 = 7.15$, $p < .05$).

The next step was to analyze whether the evaluation varied with cycle. Three major hormonal phases of the cycle were used to investigate these changes (Hawker 1984): I (days 1–5, menses); II (days 6–14, proliferative); and III (15–28, secretory). This division provides sufficient data to give a reliable measure for each data point. It was assumed (Barret and Marshall 1969) that in this division of the cycle the mean ovulation occurred at day 14 and that copulations are fertile on days 9–14. For statistical analysis a Kruskal-Wallis-One-Way Anova was applied separately to the data for both groups of females.

For the females without oral contraceptives, evaluation of the hedonic scale of androstenone changed significantly during the cycle (Kruskal-Wallis; $\chi^2 = 6.24$, $p < .05$); see Table 1. Androstenone was perceived as more pleasant around ovulation.

In contrast, the evaluation of androstenone by females with oral contraceptives did not differ significantly during cycle (Kruskal-Wallis; $\chi^2 = 2.33$; n.s.). There were differences in perceptual attitudes towards androstenone between the two groups. These differences occurred at different points in the cycle. Both groups perceived androstenone unattractively and unpleasantly.

Table 1. Hedonic Rating of Androstenone During the Female Cycle

Groups of Females		Period of Menstrual Cycle		
		I: Menses	II: Proliferative	III: Secretory
Females taking oral contraceptives	n	18	38	39
	Mean	6.3	5.4	6.3
	SD	2.8	2.7	3.1
Females taking no oral contraceptives	n	43	45	75
	Mean	6.4	7.7	6.2
	SD	2.6	3.2	2.8

However, for females taking no hormonal contraceptives this evaluation changed to a more neutral rating at mid-cycle.

A direct statistical comparison confirmed that females who were not on the pill found androstenone more attractive and pleasant than those on the pill during the proliferative period II (Kruskal-Wallis; $n = 83$; $\chi^2 = 9.95$, $p = 0.001$).

Finally the actual capacity to smell androstenone was re-examined. We divided the females into two groups: phase II females and others; or ovulating and non-ovulating females. The results showed that significantly more females at mid-cycle smelled androstenone (98.4%) then females not at mid-cycle (90.4%), $\chi^2 = 4.03$; $df = 2$; $p < .05$. This difference was not found among females who took the pill. Thus we find a different evaluation and perception of androstenone at different times of cycle, and differences between pill-users and non-pill-users perhaps due to their different hormonal status.

DISCUSSION

Rating studies are imprecise because people generally use different subjective scales and in particular associate different feelings to adjectives that describe smells. Despite this imprecision we found cyclical differences for the hedonic dimensions of attractiveness and pleasantness of androstenone. Because of the methodological difficulties in the exact determination of the ovulation these results are only suggestive. Nevertheless these findings have implications for sexual strategies, in relation to concealment of ovulation. The fact that the production of attractiveness-enhancing androstenol inevitably produces the repellent androstenone makes it difficult to propose a definite sender advantage over a non-sender. Thus a pheromonal function for both substances becomes unlikely. One male "non-smeller" could simply outreproduce another male "smeller," by approaching more females in a given time-span. This only holds if the costs of smelling are greater than the benefits reached through producing the sexual attraction enhancing androstenol. As androstenol oxidizes to androstenone the initial attractiveness becomes a repellent signal. Because this effect takes place within about 20

minutes (Labows et al. 1979), a "non-smeller" would be better off, because the repellent smell of androstenone is the long-term prevailing signal. If androstenone is a signal for females, then what advantages might "stinking" males have?

The results presented here suggest there might be a change in the emotional evaluation of males. These changes could be triggered by the reaction to androstenone. Male body odor is usually perceived as unattractive and unpleasant by females but this evaluation changes at the point in the menstrual cycle when conception is most likely. This finding is underlined by the fact that anosmia to androstenone also varies with cycle. At the conceptual optimum we find fewer anosmic females. One might suppose that smell is a basic mechanism which induces mood directly, without being filtered by other sensory input controls. Thus the change in female attitude towards male body odor could have a strong impact on mate selection and perhaps self-initiated copulations by females.

A philandering male producing androstenone would have more chances for reproduction than a nonproducing male. Non-ovulating females react negatively to the same cue. On average these males could contact more females exactly at ovulation and thus have higher chances of fertilizing. A nonproducing male would do relatively poorly in such a population.

The effects for females are more pronounced and these findings may be relevant for the discussion of the evolution of hidden estrous. Alexander and Noonan (1979) and Symons (1979) have argued that hidden estrus has evolved because females need to trick males into forming a bond. Males unaware of females' fertility would remain bonded to ensure impregnation and paternity. A female providing clues to her ovulation might risk losing male investment, due to paternal uncertainty and the limited temporal reproductive interaction.

In contrast with this line of argument, Benshoof and Thornhill (1979) and Symons (1979) have proposed a second evolutionary scenario in which hidden estrus evolved to increase the chances of successful cuckoldry by females so they "can escape the negative consequences of being pawns in marriage games" (Gray and Wolfe 1983, p. 350). Once monogamy is established, a female's best strategy would be to copulate outside the pair bond because she can then obtain superior genes with a certain expectation of paternal investment.

If we combine our findings with those of Bellis and Baker (1991), who found that extra-pair-copulations in humans peak at mid-cycle, the second hypotheses receives considerable support. With loss of estrus females are able to ensure male investment, and gain high quality males through occasional low-risk extra-pair copulations. Offspring quality could be enhanced through mate-selection and thus sperm-competition, promoted by her emotional attitude toward males at mid-cycle.

In this view both sexes would gain through the evolution of an androstenol-androstenone signaling system. Females could ensure male paternity,

while still keeping an option for extra-pair copulations which can produce genetically superior offspring, whereas males would be able to raise the probability of fertilizing a female.

This hypothesis is speculative and should be tested on further data. It seems possible that a change of attitude towards male olfactory cues may effect female choice at the point of ovulation. Thus androstenone can be called a signal—but not in the positive sense. Androstenone does not attract females, it repels them. The signal value of androstenone could lie in the fact that it might be able to change female behavior selectively.

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