Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7976–7978, October 1989
Genetics

Ability to perceive androstenone can be acquired by ostensibly anosmic people

(molecular receptor/neuronal plasticity/olfaction/specific anosmia)

CHARLES J. WYSOCKI*†‡, KATHLEEN M. DORRIES*§, AND GARY K. BEAUCHAMP*†¶

*Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104; †Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104; and ¶Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104

Communicated by Lewis Thomas, July 17, 1989

ABSTRACT **Nearly half the adult human population does not perceive an odor when sniffing androstenone (5α-androst-16-en-3-one), a volatile steroid found in human perspiration, boar saliva, some pork products (e.g., bacon), truffles, and celery. This variation in ability to perceive androstenone has a significant heritable component, suggesting that androstenone insensitivity is in part determined genetically. We now report that the ability to perceive androstenone was induced in 10 of 20 initially insensitive subjects who were systematically exposed to androstenone. Since olfactory neurons of the olfactory epithelium undergo periodic replacement from differentiating basal cells, and assuming the induction of sensitivity to be peripheral, we propose that a portion of the apparently anosmic human population does in fact possess olfactory neurons with specific receptors for androstenone. Such neurons may undergo clonal expansion, or selection of lineages with more receptors or receptors of higher affinity, in response to androstenone stimulation, much in the manner of lymphocytes responding to antigenic stimulation, thus raising odor stimulation to the level of conscious perception. As a guide to further study of the genetics and mechanism of variation of androstenone perception, we provisionally envisage three categories of human subjects, the truly anosmic, the inducible, and those subjects who either are constitutionally sensitive or have already experienced incidental induction.**

Virtually all people have selective olfactory deficits or specific anosmias (1, 2) for which the biochemical and neurophysiological foundations are unknown (3). A striking example of specific anosmia is that 40–50% of adults cannot perceive an odor when presented with androstenone (4, 5). Furthermore, detection thresholds (the lowest concentration that can be discriminated reliably) were more similar among identical twins as compared with fraternal twins (intraclass correlations were 0.95 and 0.22, respectively) and concordance for the ability to smell androstenone was considerably higher among identical than fraternal twins (100% and 61%, respectively). Hence, insensitivity to androstenone appears to have a genetic basis (6) and as such might be expected to be stable over time, as are the various forms of color blindness. However, during our research, one of us (C.J.W.), who had been insensitive to androstenone, experienced what appeared to be induced sensitivity; after months of intermittent contact with the compound, a distinct odor was detected.

METHODS

To verify this apparent shift in sensitivity to androstenone, we selected individuals who were anosmic to the compound and obtained repeated measures of threshold sensitivity to pyridine (the odor of spoiled milk), amyl acetate (the smell of pears or bananas) and androstenone (variously urinous- or musky- or sweaty- or sweet-smelling to those who detect it). The paid volunteers assigned to the experimental group individually sniffed androstenone and amyl acetate (each at the highest concentration used in the study) continuously for 3 min, three times a day, for the duration of the 6-week study. Pyridine was not systematically sniffed, but sensitivity to it was determined during weekly tests. Subjects assigned to the control group were tested weekly, but they did not sniff the odor samples between sessions.

RESULTS

Participants were androstenone-anosmic at the start of the study; hence, there was no difference between the experimental and control groups in pre-exposure androstenone sensitivity ($t_{(36)} = 1.59$, $P > 0.10$). Relative to the control group, subjects in the experimental group initially were slightly more sensitive to pyridine ($t_{(36)} = 2.55$, $P < 0.05$); this difference persisted throughout the study. The groups did not differ in sensitivity to amyl acetate ($t_{(36)} = 1.88$, $P > 0.05$). After 1 week, thresholds for androstenone decreased in the experimental group (Fig. 1). In contrast, no declines in thresholds were observed in either group for pyridine or amyl acetate.

The shift in sensitivity to androstenone was not uniform among exposed subjects: there was a bimodal distribution of changes. Half of the exposed subjects became sensitized to androstenone (Fig. 2). Subjects who did not become sensitized to androstenone did not differ from the control group across the seven tests for any of the compounds ($P > 0.15$). Among those who became sensitized, the change was both qualitative and quantitative. Sensitized individuals (those who showed a lowered threshold of ≥3 dilution steps) shifted from androstenone-anosmic to osmic; eight (80%) reported an odor quality for androstenone in session 7. Of these individuals, only one reported an odor after sniffing the blank. Among the control group, 33% described an odor quality for androstenone; however, 39% also reported an odor in response to the blank.

Further comparisons between the sensitized and nonsensitized subgroups were not revealing. The groups did not differ in their pre-exposure androstenone thresholds (session 1). The two subgroups also were indistinguishable in all tests with the other two odors. Consequently, other than by using the androstenone scores themselves (Fig. 3), we were unable to differentiate sensitized from nonsensitized individuals.

DISCUSSION

Specific anosmias may result from defective or missing molecular receptors (2). In the simplest case, a single gene might encode a receptor protein for an odor molecule or group of functionally similar odor molecules. An absent or

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MANOVA, multivariate analysis of variance.

‡To whom reprint requests should be addressed.
§Present address: Department of Psychology, Cornell University, Ithaca, NY 14853.

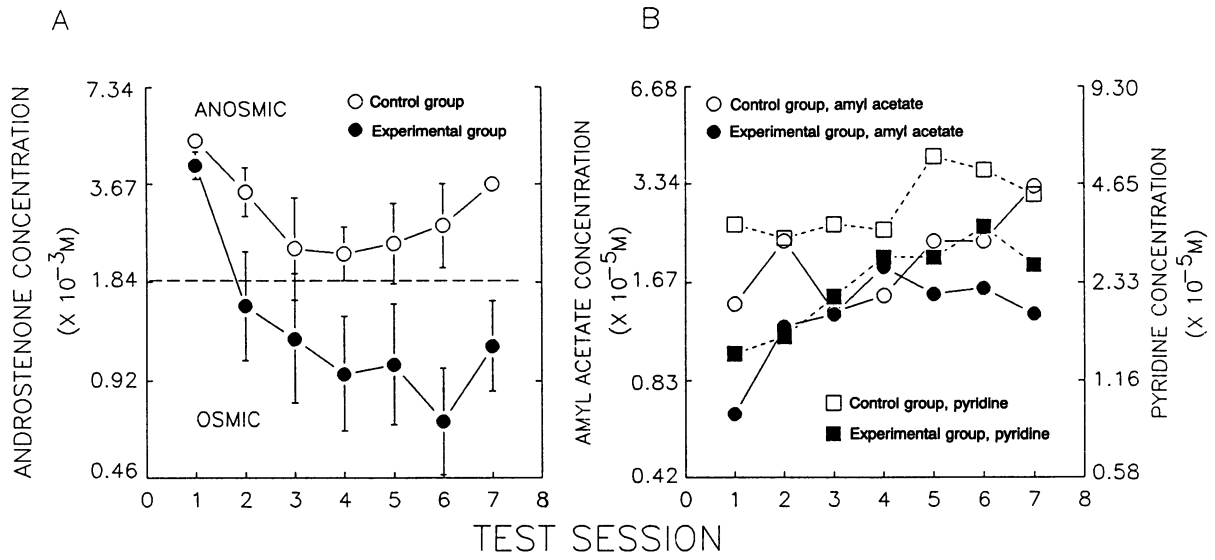

FIG. 1. Mean detection thresholds for androstenone (± SEM), amyl acetate, and pyridine. The experimental group consisted of 10 men and 10 women; the control group consisted of 9 men and 9 women. Using the methods described in Sherman *et al.* (7), pyridine was diluted from 3.72×10^{-4} M in odorless, light, white mineral oil to form 10 binary steps, 30 ml each. Androstenone and amyl acetate were diluted in 12 steps, 10 ml each, from 3.67×10^{-3} M and 1.07×10^{-3} M, respectively. Each step and its paired oil blank were presented in 300-ml polypropylene bottles. Subjects sniffed the air squeezed from each pair of bottles, one at a time, and selected the odor sample. To minimize the effects of adaptation, testing proceeded stepwise from the lowest concentration to the next highest until four (amyl acetate and pyridine) or five (androstenone) consecutive correct choices were made or until the series was exhausted; the first correct choice of the four or five was the score for that series. If the highest concentration was missed, then a score of twice the highest concentration was arbitrarily assigned. If a subject gave correct responses near the end of the series but did not reach criterion (e.g., the final two steps), then the first of those correct responses was assigned. Sessions consisted of four such ascending series for each compound; the median of the scores represented the detection threshold for each individual (for details see ref. 6). Points in the figure represent the group means of these medians. A repeated-measures multivariate analysis of variance (MANOVA) revealed a significant difference between experimental and control groups for androstenone (*A*); thresholds declined in the experimental group but not in the control group ($F_{(1,36)} = 10.13$, $P < 0.003$). Although significant variation was observed across androstenone thresholds for the control group (repeated-measures MANOVA; $F_{(6,102)} = 2.67$, $P < 0.05$), Tukey B post-hoc tests did not reveal any significant pairwise differences among the seven sessions. The dashed line in *A* represents the functional definition of androstenone anosmia. Slight increases in thresholds are apparent for amyl acetate and pyridine (*B*). To eliminate the possibility of mixing errors that might occur during multiple stimulus preparations over the length of the study, each subject was tested with a single set of stimuli in all test sessions. This may have resulted in slight changes in nominal concentrations over the entire test period.

altered gene would result in specific anosmia, which, like color blindness, should be resistant to environmental factors. Sensitivity to androstenone apparently depends upon a more subtle mechanism.

One hypothesis posits the assembly and proliferation of molecular receptors. In the immune system, rapid, elaborate, and specific responses may occur only after an initial sensitizing exposure to an antigen (8). If specific olfactory receptors are initially at subthreshold density in individuals who are anosmic to androstenone, then exposure to the compound might initiate clonal expression of receptors through an as yet unknown mechanism. The olfactory epithelium consistently replaces its neuronal receptor cells via mitotic divisions of basal stem cells (ref. 9; the estimate for complete cellular turnover among mammals is 30–45 days). Apparently, subgroups of receptor neurons form differentiated clones of their respective progenitor stem cells. By analogy with immune responses to newly encountered antigens, it may be supposed

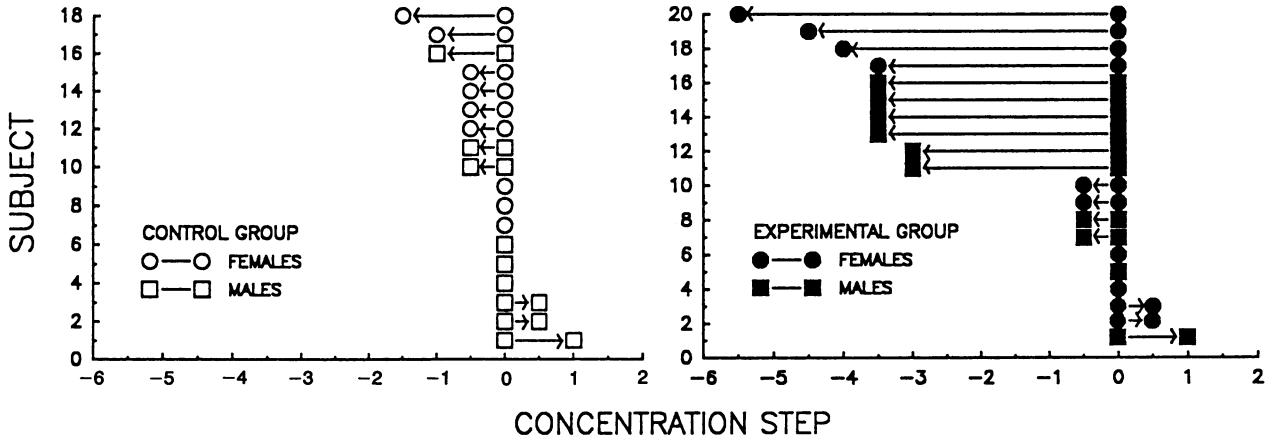

FIG. 2. Individual changes in androstenone threshold from session 1 to session 7 expressed in steps. Zero represents the individual's score at session 1 (individuals differed in their scores; hence it was not possible to express concentration steps as a measure of molarity). A shift in the negative direction indicates a decrease in threshold for detection of androstenone, i.e., increased sensitivity to the odor molecule. Half of the experimental subjects, but none of the control subjects, became sensitized (a shift of ≥3 steps; $\chi^2_{(1)} = 9.77$, $P < 0.002$, after Yates correction).

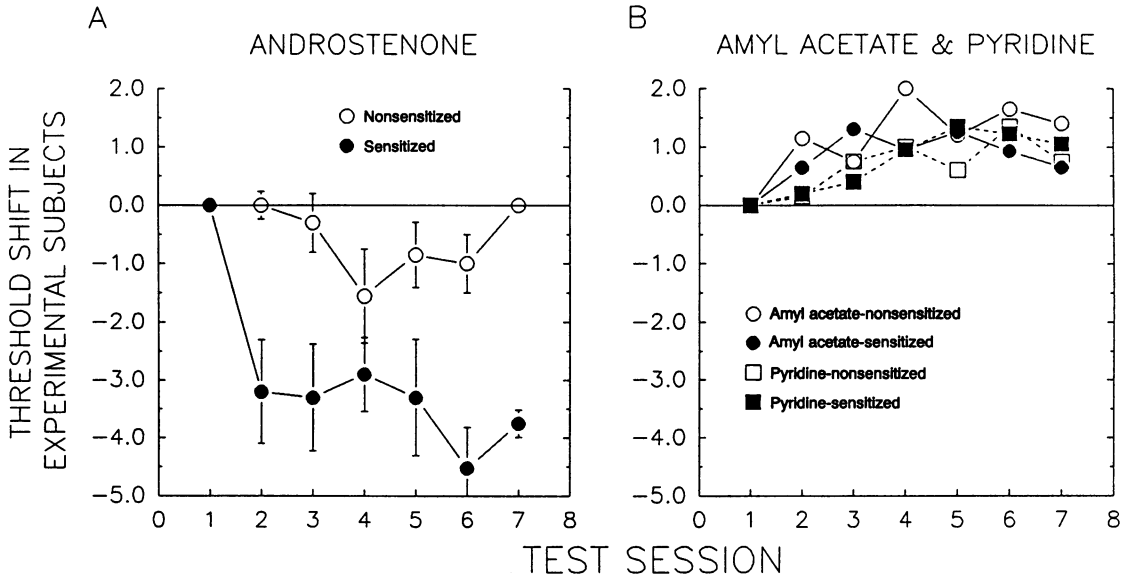

FIG. 3. Average change in thresholds from pre-exposure (session 1) for androstenone (mean ± SEM) (*A*) and amyl acetate and pyridine (mean only) (*B*) in those subjects exposed to androstenone who became sensitized to it and in those similarly exposed who did not. Individual scores were calculated as deviations from session 1 results; hence, changes are expressed in steps rather than in actual molarity. Prior to standardization, the groups did not differ in sensitivity to any compound ($P > 0.50$). A decline specific to androstenone was noted: the difference between the groups from session 2 onward was significant (repeated-measures MANOVA; $F_{(1,18)} = 33.46$, $P < 0.001$). See Fig. 1 for methods.

that exposure to the isolated odor during this continuous regenerative process stimulates proliferation of a subthreshold number of specific receptor-bearing neurons or the selection of receptor-bearing neurons of higher odor-binding affinity, raising the order of response to the level of perception. This transformation may not require the complete turnover of the epithelium; changes in sensitivity in some individuals were detected as early as 1 week after the initiation of exposure, when presumably as little as 15–25% of the epithelium would have undergone replacement.

Whether sensitivity to androstenone outlives the population of receptor cells present during sensitization remains to be determined. Preliminary data suggest that sensitivity remains for at least 6 weeks beyond termination of exposure. Furthermore, we have yet to encounter anyone who has shifted from androstenone-osmic to anosmic. If individuals remain sensitive to androstenone for extended periods beyond exposure, and if the phenomenon results from changes in the periphery, then modification of basal stem cells, perhaps via directed mutations (10), must be contemplated.

An alternative hypothesis posits more central changes. Long-term exposure of rats to an odor-free environment produces cellular atrophy in the region of the brain that receives olfactory afferents (the olfactory bulbs), which apparently results in loss of sensitivity (11, 12); exposure to odors prevents these alterations (12). Furthermore, increases in the saliency of an odor through selective exposure stimulates activity in cells of the olfactory bulbs (13). Thus, induced sensitivity to androstenone in our subjects may have resulted from new neuronal connections with and/or activity in the olfactory bulb or elsewhere in the central nervous system.

Another interpretation relies upon a shift in cognitive processes: subjects may be learning to use a more effective test-taking strategy (14, 15). This seems an unlikely explanation, since the shift in sensitivity was specific to androstenone, although members of the experimental group had equivalent experience with amyl acetate and were tested weekly with pyridine and amyl acetate as well as with androstenone.

Numerous advances notwithstanding (3), an understanding of the mechanisms underlying olfaction remains elusive. Decades ago, tests of color-blind individuals verified the Young–Helmholtz trichromatic theory of color perception (16). In studies of olfaction, a focus on people and animal models (17) with specific anosmia and further analyses of the parameters underlying perceptual shifts—e.g., time course, minimum exposure, individual differences in sensitivity, and correlations in sensitivity to other compounds—should increase our knowledge of chemosensory perception, odor quality coding, neuronal plasticity, and gene expression in neurons.

We thank Dr. George Preti of the Monell Center for confirming the purity of androstenone by combined gas chromatography/mass spectrometry and Drs. B. Cowart, J. Brand, B. Green, and R. Mason for their comments on an earlier version of this paper. This work was supported by the National Institutes of Neurological and Communicative Diseases and Stroke (NS22014 to C.J.W. and NS19616, which supports the Monell–Jefferson Chemosensory Clinical Research Center).

1. Amoore, J. E. (1971) in *Handbook of Sensory Physiology: Chemical Senses*, ed. Beidler, L. M. (Springer, New York), pp. 245–256.
2. Amoore, J. E. (1977) *Chem. Senses Flavour* **2**, 267–281.
3. Brand, J. G., Teeter, J. H., Cagan, R. H. & Kare, M. R., eds. (1989) *Receptor Events and Transduction in Taste and Olfaction* (Dekker, New York).
4. Griffiths, N. M. & Patterson, R. L. S. (1970) *J. Sci. Food Agric.* **21**, 4–6.
5. Amoore, J. E., Pelosi, P. & Forrester, L. J. (1977) *Chem. Senses Flavour* **2**, 401–425.
6. Wysocki, C. J. & Beauchamp, G. K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4899–4902.
7. Sherman, A. H., Amoore, J. E. & Weigel, V. (1979) *Otolaryngol. Head Neck Surg.* **87**, 717–733.
8. Klein, J. (1986) *Natural History of the Major Histocompatibility Complex* (Wiley-Interscience, New York).
9. Graziadei, P. P. C. & Monti-Graziadei, G. A. (1980) *J. Neurocytol.* **9**, 145–162.
10. Cairns, J., Overbaugh, J. & Miller, S. (1988) *Nature (London)* **335**, 142–145.
11. Døving, K. B. & Pinching, A. J. (1973) *Brain Res.* **52**, 115–129.
12. Laing, D. G. & Panhuber, H. (1980) *Physiol. Behav.* **25**, 555–558.
13. Wilson, D. A., Sullivan, R. M. & Leon, M. (1987) *J. Neurosci.* **7**, 3154–3162.
14. Engen, T. (1960) *Percept. Mot. Skills* **10**, 195–198.
15. Rabin, M. D. & Cain, W. S. (1986) *Percept. Psychophys.* **39**, 281–286.
16. Graham, C. H. (1965) in *Vision and Visual Perception*, ed. Graham, C. H. (Wiley, New York), pp. 414–451.
17. Wysocki, C. J., Whitney, G. & Tucker, D. (1977) *Behav. Genet.* **7**, 171–188.