Olfactory Sensitivity during the Menstrual Cycle

ROBERT G. MAIR, JANE A. BOUFFARD, AND TRYGG ENGEN
Walter Hunter Laboratory, Department of Psychology, Brown University, Providence, Rhode Island 02912

AND

THOMAS H. MORTON
Metcalf Laboratory, Department of Chemistry, Brown University, Providence, Rhode Island 02912

Received November 7, 1977

Women were tested for sensitivity to several odorants at ovulation and menstruation. Three involatile esters (pentadecalactone, coumarin, and cinnamyl butyrate) predicted by gas chromatographic data to be strongly retarded by the olfactory mucus showed similar significant changes in sensitivity. By contrast, a more volatile ester (amyl acetate) predicted to diffuse more readily though the mucus showed no such changes. This suggests that variations in olfactory sensitivity observed between ovulation and menstruation depend on odorant volatility and thus may result from peripheral mechanisms limiting the access of odorant molecules to the olfactory receptors.

Olfactory sensitivity in women for the musk pentadecalactone (also known as Exaltolide or Thibetolide) varies with phase of the menstrual cycle, being highest around ovulation and lowest around menses (Good, Geary, & Engen, 1976; Le Magnen, 1948; Vierling & Rock, 1967). This change in sensitivity has been related to the level of circulating estrogen (Good *et al.*, 1976; Le Magnen, 1950; Schneider, Costiloe, Howard, & Wolf, 1958). Sensitivity for other odorants, notably methyl salicylate, safrole, pyridine (Meixner, Note 1), citral (Schneider & Wolf, 1955), *m*-xylene (Köster, 1965), oil of cloves, *n*-butanol, and amyl acetate (Kahn, Note 2), has been reported not to vary or to vary to a lesser extent. Pietras and Moulton (1974, 1975) have observed that rats show cyclic variations in their sensitivities for cyclopentanone, eugenol, and α-ionone related to circulating concentrations of ovarian steroids. Henkin and Bartter (1966) have demonstrated that untreated patients with hypoadrenocortical functioning show markedly increased olfactory sensitivity. Normal olfactory sensitivity was reported in these patients 2 to 3 days after administration of glucocorticoids.

How do circulating hormones affect olfactory sensitivity? Schneider and Wolf (1960) have suggested that local changes in the nasal mucosa, affecting the constriction of nasal airways and thus the flow of odorant molecules to the receptors, may be the basis of estrogen-related changes in olfactory acuity. Although the olfactory mucosa is inaccessible to direct study, menstruation has been associated with engorgement of nasal cavernous tissue, increased nasal secretion, and congestion. There are several arguments against this position. First, Pfaff and Pfaffmann (1969) have shown that testosterone-induced changes in olfactory bulb activity cannot be accounted for by gross changes in nasal air flow. Second, Pietras and Moulton (1974, 1975) report that bilateral cervical

0363-3799/78/0022-0090$02.00/0

sympathectomy does not affect the cyclic variations in olfactory sensitivity for female rats, indicating that circulating hormones do not control olfactory sensitivity by acting on the autonomic nervous system to affect the engorgement of vascularized tissue and secretions of nasal glands. Third, such an explanation does not account for the observation that a more robust and consistent change has been seen for pentadecalactone than for other odorants.

Several investigators have suggested that steroid hormones affect olfactory sensitivity by acting on the brain and controlling the overall excitability of the olfactory system (cf. Pfaff & Pfaffmann, 1969; Pietras & Moulton, 1974, 1975). Units in the preoptic area of castrated male rats show generally increased responsiveness to odors and to electrical stimulation of the olfactory bulb following direct preoptic administration of testosterone (Pfaff & Pfaffmann, 1969). Pfaff and Gregory (1971) report that this androgen-related sensitivity change does not alter the relative responsiveness of preoptic units to estrus as compared to ovariectomized female urine odors or to urine as compared to nonurine odors. This indicates a lack of specificity, even for biologically salient odors, in the hormonally controlled sensitivity changes at the preoptic level. Such a mechanism can account for an overall change in olfactory sensitivity; however, it provides no compelling explanation for a specific sensitivity change such as that reported for pentadecalactone.

Another hypothesis is that circulating hormones act directly on the glands producing olfactory mucus, controlling their rate of secretion and thus the effective barrier through which odor molecules must diffuse to reach the receptor sites. Ebling (1974, 1977) has described a similar mechanism for direct hormonal control over secretory activity of sebaceous and apocrine glands, in which testosterone stimulates and estrogen inhibits secretions. Fortunato and Bindoni (1971) report a strong reduction of acid mucopolysaccharides in the olfactory mucus layer following hypophysectomy in rats. As acid mucopolysaccharides in the mucus are thought to originate from olfactory supporting cells and, possibly, Bowman's glands (cf. Bronshtein & Leont'ev, 1973) this supports an argument for hormonal control over secretion of the olfactory mucus layer.

Mozell and his co-workers have suggested that odorant molecules differ in their propensity to sorb onto the olfactory mucus layer and that these differences affect the spatial and temporal distribution of neural activity elicited across the olfactory epithelium (Hornung, Lansing, & Mozell, 1975; Mozell, 1964a, 1964b, 1967). Mozell (1970) and Mozell and Jagodowicz (1973, 1974) have shown a correlation between these putative differences and retention time on a gas chromatographic column having a polar (Carbowax 20M) stationary phase. The present study examines the extent to which gas chromatography (GLPC) can predict olfactory properties of pure organic compounds.

For a given set of GLPC conditions, a compound's retention time is largely a function of its boiling point and its polarity. All odorants for which little or no variation in sensitivity has been reported are more volatile and have much shorter retention times on a Carbowax 20M column than does pentadecalactone (see Table 1). We surmise two tendencies that odorants having substantially longer GLPC retention times should exhibit in relation to their distribution in

olfactory mucus. First, as Mozell has suggested, they should show steeper concentration gradients across the olfactory epithelium. Second, as they tend to be large, polar molecules, mucus should retard their approach to the receptors. A GLPC analogy suggests an explanation for the specificity observed in cyclic olfactory sensitivity changes.

The aim of this investigation is to compare changes in olfactory sensitivity observed for pentadecalactone with sensitivities for odorants predicted by GLPC analogy to have similar sorptive properties. Two esters, coumarin and cinnamyl butyrate, with approximately the same boiling point as pentadecalactone (~300°C) were chosen. Amyl acetate, an ester with a much lower boiling point, was chosen as a control. Pentadecalactone, coumarin, and cinnamyl butyrate have much longer retention times on a Carbowax 20M column than does amyl acetate (see Table 1). Changes in olfactory sensitivity between ovulation and menstruation for pentadecalactone were compared with changes in sensitivity for coumarin and cinnamyl butyrate, which should have similar sorptive properties, and with amyl acetate, which should be retarded by the mucus to a much lesser extent.

METHODOLOGY

Subjects. Subjects were 12 adult women, ranging in age from 20 to 32 years. All reported to be in good health and had regular menstrual cycles with average lengths varying from 26 to 32 days. None had a history of menstrual or hormonal dysfunction, and none was taking birth control pills. Menstrual histories were taken from each subject, and presumed time of ovulation was determined by extrapolation from these data and by monitoring basal temperature. Each subject was tested at least twice, once during ovulation and once during menstruation. As the purpose of this study is to compare the sensitivity change observed for pentadecalactone with those observed for other odorants, subjects were tested on as many as 3 successive days around the presumed time of ovulation until a clear

TABLE 1
GLPC Retention Times on a Carbowax 20M Column for Odorants[a]

Odorant	Retention time (sec)
Coumarin	888
Pentadecalactone	600
Cinnamyl butyrate	546
Methyl anthranilate	408
Oil of cloves	315
Methyl salicylate	180
Safrol	150
Phenethyl alcohol	135
Citral	105
n-Butanol	45
Amyl acetate	35
m-Xylene	35
Pyridine	35

[a] GLPC analyses were performed on a 6-ft × 0.125-in. 16.7% Carbowax 20M on 45–60 Chromosorb W column in a Perkin–Elmer Fll gas chromatograph with a flame ionization detector. Retention times were measured at a column temperature of 220°C and a carrier gas flow of 30 ml/min.

increase in sensitivity was observed for pentadecalactone. One subject in each part of the experiment was excluded showing no change in sensitivity for pentadecalactone on any days tested. In each part of the experiment three subjects were tested first at ovulation, and three were tested first at menstruation.

Odorants. The four odorants used in this study are all esters; purity was checked by GLPC. Cinnamyl butyrate (Givaudan) was purified of a volatile, fragrant impurity by preparative GLPC before use; the other three compounds were used from the bottles without further purification. Odorants were diluted in dibutyl pthalate and presented in equilibrium sniffers (cf. Engen, Kilduff, & Rummo, 1975) containing either 40 ml of pure solvent or a solution of odorant. Concentrations of the odorant solutions determined in a pilot experiment as being slightly above threshold when sampled in the sniffers were 1.6 mM for pentadecalactone, 0.96 mM for coumarin, 0.78 mM for cinnamyl butyrate, and 0.038 mM for amyl acetate.

Experimental procedure. The experiment was divided into two parts. Six subjects participated in the first part in which pentadecalactone, cinnamyl butyrate, and coumarin were the odorants. Six other subjects participated in the second part using amyl acetate and pentadecalactone as odorants. In both parts, sensitivity for each odorant was tested in a separate block of trials and the order in which these were presented was counterbalanced across subjects. Each block consisted of 60 trials, a random sequence of 30 trials with the sniffer containing the solution of odorant, and 30 trials with only the solvent. Subjects were instructed and practiced in sniffing the odorant in a consistent manner and then were blindfolded for the duration of the experiment. Prior to each block of test trials, the subjects were familiarized with the target odor in a series of practice trials. On each trial, the experimenter uncovered the sniffer and placed it under the subject's nose for sampling. The subject's task was to say whether pure solvent or a solution of odorant was presented, upon which the experimenter would state the correct answer. Subjects were tested in an environmental chamber that is part of the laboratory olfactometer. They abstained from eating and smoking for at least 2 hr before testing and were not tested on days they reported respiratory problems.

RESULTS

The results are reported both in terms of the number of correct responses in each block of trials and of d', an index of sensory sensitivity presumed to be free of response bias (Engen, 1971). Figure 1 depicts the number of correct responses for odorants in both parts of the experiment. Performance during menstruation is plotted along the abscissa, and performance during ovulation along the ordinate. Points lying above the solid line indicate that the subjects made more correct responses during ovulation than during menstruation. All subjects made more correct responses during ovulation for pentadecalactone, cinnamyl butyrate, and coumarin. Two subjects made more and four subjects made fewer correct responses during ovulation than during menstruation for amyl acetate. An examination of Fig. 1 indicates that there is a considerable degree of overlap in performance for the four odorants during both menstrual conditions. An analysis of the number of correct responses for the first part of the experiment, using a two-factor analysis of variance with repeated measures (Keppel, 1973), shows that

sensitivity to the three odorants, pentadecalactone, cinnamyl butyrate, and coumarin, was significantly higher at ovulation [$F(1,5) = 95.33, p < .001$]. There was no significant difference between them [$F(1,5) = 2.74$], nor was there a significant interaction between menstrual condition and odorants [$F(2,10) = 1.62$]. An analysis of the number of correct responses for the second part of the experiment with amyl acetate and pentadecalactone showed that sensitivity was significantly higher at ovulation [$F(1,5) = 19.25, p < .01$]. Again there was no significant difference between the odorants [$F(1,5) = 0.49$], but this time there was a significant interaction between the odorants and menstrual condition [$F(1,5) = 27.25, p < .01$], indicating that pentadecalactone was affected more by menstrual condition than amyl acetate.

The analysis of the data in terms of d' for individual subjects in both parts of the experiment is presented in Table 2. All subjects showed a higher d' in the ovulatory condition for pentadecalactone, cinnamyl butyrate, and coumarin. Two subjects showed a higher and four a lower d' in the ovulatory condition for amyl acetate. In Part 1 sensitivity at ovulation was significantly higher for pentadecalactone ($t = 16.32, p < .01$), coumarin ($t = 4.88, p < .01$) and cinnamyl butyrate ($t = 4.47, p < .01$). In Part 2 sensitivity at ovulation was significantly higher for pentadecalactone ($t = 4.72, p < .01$) but not for amyl acetate ($t = -1.10$).

DISCUSSION

The results show that sensitivity for pentadecalactone, cinnamyl butyrate, and coumarin is significantly higher at ovulation, whereas sensitivity for amyl acetate appears unchanged. The lack of significant difference in the number of correct responses between the three test odorants in Part 1 and the two test odorants in Part 2 indicates that they were well matched for intensity. The lack of significant interaction for the number of correct responses among the three test odorants as a function of menstrual condition in Part 1 indicates that the change in sensitivity observed for pentadecalactone was not significantly different from that observed for cinnamyl butyrate and coumarin. An examination of Fig. 1 supports this con-

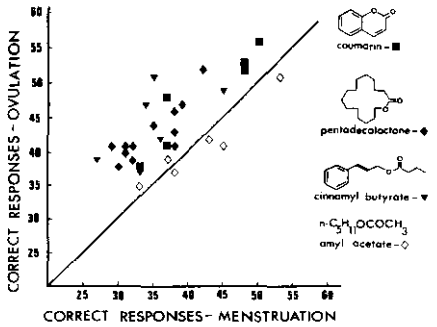

FIG. 1. Number of correct responses during ovulation plotted against the number of correct responses during menstruation for each subject. Points lying above the solid line indicate that the subject made more correct responses during ovulation than during menstruation. All subjects made more correct responses for pentadecalactone (closed diamonds), cinnamyl butyrate (closed triangles), and coumarin (closed squares). Two subjects made more and four made fewer correct responses during ovulation for amyl acetate (open diamonds).

TABLE 2
VALUES OBTAINED FOR d' FOR INDIVIDUAL SUBJECTS AT OVULATION (O) AND MENSTRUATION (M)

		Subject no.					
		1	2	3	4	5	6
Part 1							
Pentadecalactone	O	1.01	0.92	1.46	1.27	1.80	0.81
	M	0.08	0.09	0.70	0.46	0.81	0.17
Coumarin	O	0.95	2.22	0.70	3.12	2.68	1.68
	M	0.65	1.68	0.28	2.12	2.18	0.60
Cinnamyl butyrate	O	2.12	1.05	1.63	2.27	0.60	0.79
	M	0.42	0.54	0.49	1.36	0.35	−0.26
		7	8	9	10	11	12
Part 2							
Pentadecalactone	O	0.95	0.67	2.57	0.97	1.28	0.98
	M	−0.09	0	1.07	0.18	0.70	0.70
Amyl acetate	O	0.98	0.67	0.42	0.60	2.46	1.09
	M	1.36	0.60	0.25	0.70	2.68	1.16

clusion. The significant interaction for the number of correct responses between the two test odorants and menstrual condition in Part 2 of the experiment indicates that there is a significant difference in the sensitivity change observed for amyl acetate and pentadecalactone. This conclusion is also supported by the analysis of d'.

The observed sensitivity changes for pentadecalactone, cinnamyl butyrate, and coumarin are similar and substantially greater than any observed for amyl acetate. Any proposed mechanism for hormonal control of olfactory sensitivity must account for this specificity. It is possible that some common stereochemical trait or biological significance relating pentadecalactone, coumarin, and cinnamyl butyrate results in their exciting a class of receptors or neurons at some higher brain center that is distinct from those responding to amyl acetate. If this is the case, then it is conceivable that some differential change in neural excitability could account for the present results. This seems unlikely for several reasons. First, all four odorants are esters. Second, even given the relative unimportance of functional groups in determining the odor quality of large molecules, the molecular shapes of pentadecalactone, coumarin, and cinnamyl butyrate do not suggest any clear stereochemical relationship that could serve to distinguish them from amyl acetate. Third, there is no evidence for either coumarin or cinnamyl butyrate having any biological association with pentadecalactone that might relate them in terms of functional significance.

The most apparent chemical differences between amyl acetate and the other odorants are amyl acetate's smaller molecular weight and higher volatility. According to Stuiver (Note 3), access of odorant molecules to the olfactory receptors depends on (i) the volume of nasal airflow diverted to the olfactory cleft, (ii) the proportion of odorant molecules absorbed from that airstream into respiratory mucus before reaching the olfactory epithelium, and (iii) the propen-

sity of odorant molecules reaching the olfactory epithelium to absorb into and migrate through the olfactory mucus. Although gross changes in nasal airflow have been effectively ruled out as the basis for hormonally induced sensitivity changes, alterations in the sorptive properties of the respiratory or the olfactory mucus remain as viable hypotheses. An increase in the propensity of the respiratory mucus to absorb odorant molecules would result in fewer molecules being distributed to the olfactory epithelium. It is conceivable that hormonally induced changes in the sorptive properties of the respiratory mucus could discriminate between molecules on the basis of molecular size or volatility and thus account for the present results. So little is known concerning the absorption of odorant molecules into the respiratory mucus that no strong arguments can be raised for or against this possibility.

A more plausible interpretation of the present results is that changes in olfactory sensitivity during the menstrual cycle are related to hormonally controlled changes in the olfactory mucus layer. Mucus should retard the passage of involatile odorants more than volatile ones, and we infer that GLPC retention time measures this retarding effect. We surmise two means by which an increase in mucus thickness can affect sensitivity to weak stimuli. One involves a spatial variation: The effective stimulating area along the olfactory epithelium is decreased because the concentration, c, of odorant molecules is diminished at more remote receptor sites. This could happen, for example, if odorant molecules were preferentially deposited in regions where there are few or no chemoreceptors. As thickness of mucus increases, competition between sorption in sensitive areas and sorption in insensitive areas might shift more toward the latter. The other involves a temporal variation, hypothesizing that receptor response to weak stimuli is a function of the rate of change of the concentration, dc/dt, and that this slope decreases in much the same fashion as GLPC peaks become broader as a function of their retention times.

Either model predicts that an intermediate variation of sensitivity with the menstrual cycle should be observed for some odorants with faster diffusion rates than cinnamyl butyrate and slower rates than amyl acetate. Methyl anthranilate is an ester whose boiling point is nearly as high as that of pentadecalactone, but whose GLPC retention time lies between that of cinnamyl butyrate and amyl acetate (see Table 1). In tests of five female subjects three showed greater sensitivity at ovulation, one at menstruation, ane one showed no difference. Although more subjects need to be studied more thoroughly before significance can be attached to these particular results, the preliminary data are consistent with a GLPC analogy.

REFERENCES

BRONSHTEIN, A. A., & LEONT'EV, V. G. The sodium and potassium content of the mucus of the olfactory epithelium of vertebrates. *Journal of Evolutionary Biochemistry and Physiology*, 1973, 8, 520–524.

EBLING, F. J. Hormonal control and methods of measuring sebaceous gland activity. *The Journal of Investigative Dermatology*, 1974, 62, 161–171.

EBLING, F. J. Hormonal control of mammalian skin glands. In D. Müller-Schwarze and M. M. Mozell (Eds.), *Chemical signals in vertebrates*. New York: Plenum Press, 1977. Pp. 17–34.

ENGEN, T. Olfactory psychophysics. In L. M. Beidler (Ed.), *Handbook of sensory physiology*. New York: Springer-Verlag, 1971. Vol. 4, pp. 216–244.

ENGEN, T., KILDUFF, R., & RUMMO, N. The influence of alcohol on odor detection. *Chemical Senses and Flavor*, 1975, 1, 323–329.

FORTUNATO, V., & BINDONI, M. Effects of hypophysectomy on the secretion of the olfactory mucosa in the rat. *Clinica Otorinolaringoiatrica*. 1971, **23**, 131–136.

GOOD, P. R., GEARY, N., & ENGEN, T. The effect of estrogen on odor detection. *Chemical Senses and Flavor*, 1976, **2**, 45–50.

HENKIN, R. I., & BARTTER, F. C. Studies on olfactory thresholds in normal man and in patients with adrenal cortical insufficiency: The role of adrenal cortical steroids and of serum sodium levels. *Journal of Clinical Investigation*, 1966, **45**, 1631–1639.

HORNUNG, D. E., LANSING, R. D., & MOZELL, M. M. Distribution of butanol molecules across bullfrog olfactory mucosa. *Nature (London)*, 1975, **254**, 617–618.

KEPPEL, G. *Design and analysis: A researcher's handbook*. Englewood Cliffs, NJ: Prentice-Hall, 1973.

KÖSTER, E. P. Olfactory sensitivity and the menstrual cycle. *International Rhinology*, 1965, **3**, 57–64.

LE MAGNEN, J. Un cas de sensibilité olfactive se présentant comme un caractère sexuel secondaire feminine. *Comptes Rendus de l'Académie des Sciences, Paris*, 1948, **226**, 694.

LE MAGNEN, J. Nouvelles données sur le phénomène de l'exaltolide. *Comptes Rendus de l'Académie des Science, Paris*, 1950, **228**, 497.

MOZELL, M. M. Olfactory discrimination: Electrophysiological spatiotemporal basis. *Science*, 1964a, **143**, 1336–1337.

MOZELL, M. M. Evidence for sorption as a mechanism of the olfactory analysis of vapors. *Nature (London)*, 1964b, **203**, 1181–1182.

MOZELL, M. M. The effect of concentration upon the spatiotemporal coding of odorants. In T. Hayashi (Ed.), *Olfaction and taste II*. Oxford: Pergamon Press, 1967. Pp. 117–124.

MOZELL, M. M. Evidence for a chromatographic model of olfaction. *Journal of General Physiology*, 1970, **56**, 46–63.

MOZELL, M. M., & JAGODOWICZ, M. Chromatographic separation of odorants by the nose: Retention times measured across in vivo olfactory mucosa. *Science*, 1973, **181**, 1247–1249.

MOZELL, M. M., & JAGODOWICZ, M. Mechanisms underlying the analysis of odorant quality at the level of the olfactory mucosa. part 1. Spatiotemporal sorption patterns. *Annals of the New York Academy of Science*, 1974, **237**, 76–90.

PFAFF, D., & GREGORY, E. Olfactory coding in olfactory bulb and medial forebrain bundle of normal and castrated male rats. *Journal of Neurophysiology*, 1971, **34**, 208–216.

PFAFF, D., & PFAFFMANN, C. Olfactory and hormonal influences on the basal forebrain of the male rat. *Brain Research*, 1969, **15**, 137–156.

PIETRAS, R., & MOULTON, D. Hormonal influences on odor detection in rats: Changes associated with the estrus cycle, pseudopregnancy, ovariectomy, and administration of testosterone propionate. *Physiology and Behavior*, 1974, **12**, 475–491.

PIETRAS, R., & MOULTON, D. Effects of gonadal steroids on odor detection performance in the rat. In D. Denton and J. Coghlan (Eds.), *Olfaction and taste V*. New York: Academic Press, 1975. Pp. 403–409.

SCHNEIDER, R., COSTILOE, P., HOWARD, R., & WOLF, S. Olfactory perception thresholds in hypogonadal women: Changes accompanying the administration of androgen and estrogen. *Journal of Clinical Endocrinology and Metabolism*, 1958, **18**, 379–390.

SCHNEIDER, R., & WOLF, S. Olfactory perception thresholds for citral utilizing a new type of olfactorium. *Journal of Applied Physiology*, 1955, **8**, 337–350.

SCHNEIDER, R., & WOLF, S. Relation of olfactory acuity to nasal membrane function. *Journal of Applied Physiology*, 1960, **15**, 914–920.

VIERLING, J., & ROCK, J. Variations in olfactory sensitivity to exaltolide during the menstrual cycle. *Journal of Applied Physiology*, 1967, **22**, 331–351.

REFERENCE NOTES

1. MEIXNER, C. *Changes in olfactory sensitivity during the menstrual cycle.* Unpublished M. A. Thesis, Brown University, Providence, Rhode Island, 1955.
2. KAHN, R. *Sensory threshold fluctuation as a function of menstrual cycle phase.* Unpublished dissertation, Yeshiva University, New York, 1965.
3. STUIVER, M. *Biophysics of the sense of smell.* Unpublished dissertation, University of Groningen, Groningen, Holland, 1958.