activator. Therefore, there should be inhibitory factors to control over-expression of TGase activity. ATP is a candidate for such a function, since it inhibits TGase activity at physiological concentrations. Moreover, intracellular ATP concentration is related to the physiological state of cells.

1 Folk, J. E., A. Rev. Biochem. *49* (1980) 517.
2 Pisano, J. J., Finlayson, J. S., and Peyton, M. P., Biochemistry *8* (1969) 871.
3 Harding, H. W., and Rogers, G. E., Biochemistry *10* (1971) 624.
4 Rice, R. H., and Green, H., Cell *11* (1977) 417.
5 Rice, R. H., and Green, H., Cell *18* (1979) 681.
6 Williams-Ashman, H. W., Notides, A. C., Pabalan, S. S., and Lorand, L., Proc. natl Acad. Sci. USA *69* (1972) 2322.
7 Maxfield, F. R., Willingham, M. C., Davies, P. J., and Pastan, I., Nature (Lond.) *277* (1979) 661.
8 Davies, P. J. A., Davies, D. R., Levitzki, A., Maxfield, F. R., Milhaud, P., Willingham, M. C., and Pastan, I. H., Nature (Lond.) *283* (1980) 162.
9 Lorand, L., Weissmann, L. B., Epel, D. L., and Bruner-Lorand, J., Proc. natl Acad. Sci. USA *73* (1976) 4479.
10 Siefring Jr., G. E., Apostol, A. B., Velasco, P. T., and Lorand, L., Biochemistry *17* (1978) 2598.
11 Birckbichler, P. J., and Patterson Jr, M. K., Annls N.Y. Acad. Sci. *312* (1978) 354.
12 Yupsa, S. H., Ben, T., and Hennings, H., Carcinogenesis *4* (1983) 1413.
13 Loewy, A. G., and Matacic, S. S., Biochim. biophys. Acta *668* (1981) 167.
14 Loewy, A. G., Matacic, S. S., Rice, P., and Stern, J., Biochim. biophys. Acta *668* (1981) 177.
15 Cohen, I., Blankenberg, T. A., Borden, D., Kahn, D. R., and Veis, A., Biochim. biophys. Acta *628* (1980) 365.
16 Laemmli, U. K., Nature (Lond.) *227* (1970) 680.
17 Lorand, L., Campbell-Wilkes, L. K., and Cooperstein, L., Analyt. Biochem. *50* (1972) 623.
18 Brenner, S. C., and Wold, F., Biochim. biophys. Acta *522* (1978) 74.
19 Bergamini, C. M., Signorini, M., and Poltronieri, L., Biochim. biophys. Acta *916* (1987) 149.
20 Achyuthan, K. E., and Greenberg, C. S., J. biol. Chem. *262* (1987) 1901.
21 Birckbichler, P. J., Carter, H. A., Orr, G. R., Conway, E., and Patterson Jr, M. K., Biochem. biophys. Res. Commun. *84* (1978) 232.
22 Milhaud, P. G., Davies, P. J. A., Pastan, I., and Gottesman, M. M., Biochim. biophys. Acta *630* (1980) 476.
23 Birckbichler, P. J., Orr, G. R., Patterson Jr, M. K., Conway, E., and Carter, H. A., Proc. natl Acad. Sci. USA *78* (1981) 5005.
24 Scott, K. F. F., Meyskens Jr, F. L., and Russell, D. H., Proc. natl Acad. Sci. USA *79* (1982) 4093.

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Changes in olfactory perception during the menstrual cycle

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Summary. The aim of the study was to find correlations between changes in olfactory sensitivity and the menstrual cycle. 14 young, healthy volunteers participated in the experiments. Subjects menstruated regularly and did not use oral contraceptives. Three odorants were investigated: phenylethyl alcohol, androstenone, and nicotine. Dilution series of the odorants were prepared, and presented to the subjects in order to determine the detection thresholds (triple forced choice). Additionally, the subjects' hedonic estimates of the odorants were measured, and mood states as well as hormonal levels of LH and estrogen were determined. Before the actual experiments started, subjects participated in three training sessions.

One experiment was subdivided into 5 phases (two pre- and two postovulatory phases; one ovulatory phase). Only with regard to androstenone did trend analyses reveal a significant quadratic relationship between hedonic estimates and phases of the menstrual cycle, peaking at ovulation. Olfactory sensitivity was not significantly influenced by the menstrual cycle.

Key words. Menstrual cycle; olfaction; threshold; hormone; hedonic estimates; mood; intensity estimates.

Changes in olfactory sensitivity in the course of the menstrual cycle have been reported in several studies[1–3]. It was observed that the highest degree of olfactory sensitivity coincided with ovulation. These findings, however, were not received without dissent. Amoore et al.[4], Kloek[5] and Henkin (cited from Vierling and Rock[3]) did not find changes in olfactory sensitivity during the menstrual cycle. The reason for this discrepancy might be the fact that these groups did not determine the serum levels of hormones, the establishing of which would have fixed the exact time of ovulation. Moreover, of all these teams only Vierling and Rock[3] and Amoore et al.[4] submitted their data to statistical analyses.

Recently, Doty et al.[6] were able to confirm the findings of Le Magnen[1]. Within the scope of a signal-detection-paradigm, they statistically determined two peaks of ol-

factory sensitivity. After having established the serum level of hormones, they showed that these peaks occurred during the midcycle or the midluteal phase.

In the studies mentioned above, with the exception of Köster[2] (metaxylene) and Doty et al.[6] (furfural), all experimenters used exaltolide, a synthetic musk, as the stimulant. It was only Le Magnen[1] who, besides exaltolide, presented other odorants as well (safrol, pyridine and guaiacol). In his experiments he observed changes in detection thresholds only for musky odors. These data were also not submitted to statistical analyses.

In these experiments only the olfactory sensitivity was investigated. Little or no attention was payed to either the emotional or the cognitive aspect of smelling. Thus, the aims of the present study were not only to determine the detection thresholds, but also to assess the subjects' hedonic ratings of odorants at different phases of the menstrual cycle. In addition to a musky odorant (androstenone), two other odorous substances (phenylethyl alcohol, nicotine) were used as stimulants, in order to distinguish between specific effects evoked by the musky odor and nonspecific changes in olfactory sensitivity.

Additionally, the emotional state of the subjects at different times of the cycle was recorded. Thereby, it was possible to distinguish between specific changes in the hedonic ratings of the presented odorants and general changes in the emotional state. The phases of the cycle and, in particular, the exact time of ovulation, were established by measuring the serum levels of estrogen and luteinizing hormones.

Material and methods

35 healthy volunteers participated in the study, none of whom used oral contraceptives. All subjects gave written informed consent according to the declaration of Helsinki/Tokyo/Venice. Data of 21 subjects were excluded from analyses for diverse reasons (anovulatory cycle, length of cycle more than 30 days, ovulation after the 20th day of cycle, discontinuance of measurements for private reasons or for reasons of health). Menstrual cycles of the remaining 14 subjects (mean age 26.6 years) were regular (mean cycle length: 28 days; mean day of ovulation: day 14). Measurements were obtained during the entire course of one cycle.

Three odorous substances were used as the stimulants: androstenone (5-α-androst-16-en-3-one, Schering), phenylethyl alcohol (2-phenylethanol, Fluka), and nicotine (S-(−)-nicotine, Brinkmann). Androstenone and phenylethyl alcohol were dissolved in odorless propylene glycol (1,2-propanediol, Fluka), nicotine was diluted with double-distilled water. Maximum concentrations of the solutions were 1.44 mmol/l for androstenone, 4130 mmol/l for phenylethyl alcohol, and 151 mmol/l for nicotine.

Dilutions were prepared successively by adding 40 ml of the solvent to 40 ml of the preceding solution. The solutions were stored in polypropylene bottles (volume 250 ml), and kept in a ventilated cabinet.

Measurements were obtained at the same time of day (± 1 h). Subjects were requested not to smoke and to avoid strong olfactory stimuli such as peppermint or menthol for at least 1 h before the experiments started. They were also asked not to use perfume during the experiments. Duration of one measuring session was approximately 30 min.

One week before menstruation, subjects participated in three adaptational sessions in which they were made familiar with the experimental procedures. From the 1st until the 10th day of the cycle measurements were made every 2nd or 3rd day. From the 10th day of the cycle until the 2nd day after ovulation measures were obtained daily. After that until the end of the cycle measurements were again obtained every 2nd or 3rd day. This was necessary in order not to inconvenience the subjects too greatly (regular appearance at the laboratory, venipuncture, etc.).

The experiments were conducted in a quiet, air-conditioned room. During the experiments, subjects put on opaque glasses. In order to avoid contamination by handling the bottles containing the odorants, the experimenters wore odorless, plastic disposable gloves.

The following parameters were measured: detection thresholds, hedonic ratings of the three odorants, the subjects' emotional state, and the serum levels of estrogen and luteinizing hormones. All experimental sessions started with the subjects' completing a questionnaire, in order to obtain information concerning their mood state. The underlying principle of the questionnaire[7] was that of semantic differentials. The questionnaire contained 16 pairs of items (e.g., tense – relaxed).

Subsequently, thresholds were determined by employing the method introduced by Wysocki and Beauchamp[8]. Three flasks, of which two contained the solvent and one contained the odorant at a certain dilution, were presented to each subject in a randomized order. The flasks were gently squeezed in front of the subjects' nostrils. Subjects had to indicate the bottle which they presumed to contain the odorant. Within the scope of this triple-forced-choice task, the odorants, in rising concentrations, were presented to the subjects every 20 s, until they had correctly discerned the solutions three times in succession. The lowest of these three concentrations was defined as the detection threshold. This procedure was performed twice for all three odorants. Subsequently, the mean of both threshold values was calculated. The sequence in which the odors were presented was always the same for one subject, but was randomized over all participants. During the experimental sessions, subjects had no feedback as to the accuracy of their decision.

Subsequently, subjects smelled stock solutions of the odorants and rated their hedonic quality using a visual analog scale (left extreme = 50 U: extremely pleasant; right extreme = − 50 U: extremely unpleasant). Finally,

Means (M) and standard deviations (SD) of measured parameters

Thresholds. An increase in the dilution steps indicates a rise in olfactory sensitivity (triple-forced-choice method).
Hedonic estimates. Negative numbers represent unpleasant sensations, positive numbers represent pleasant sensations (50 U: extremely pleasant; – 50 U: extremely unpleasant).
Mood. Higher numbers indicated an elevation in mood. The underlying principle of the questionnaire was semantic differentials. The questionnaire contained 16 pairs of items (e.g., tense – relaxed).

		Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Thresholds [dilution steps]						
Androstenone	M:	−7.94	−7.66	−8.39	−8.30	−7.29
	SD:	2.41	2.50	4.52	2.57	2.94
Nicotine	M:	−8.46	−9.00	−8.86	−8.51	−9.07
	SD:	3.59	3.15	2.82	2.71	3.31
Phenylethylalcohol	M:	−15.25	−14.17	−13.34	−13.02	−13.92
	SD:	5.37	4.08	2.45	2.61	3.62
Hedonic estimates [U]						
Androstenone	M:	−13.48	−7.84	−4.14	−6.18	−6.79
	SD:	12.81	15.82	16.38	14.72	16.82
Nicotine	M:	−14.39	−12.22	−13.47	−13.55	−13.74
	SD:	12.35	10.70	7.83	9.56	13.88
Phenylethylalcohol	M:	17.91	17.06	16.87	18.41	16.58
	SD:	11.67	12.28	15.01	13.93	14.77
Mood [U]	M:	77.37	81.57	82.32	79.45	78.28
	SD:	12.25	12.38	9.73	13.11	11.60
Estrogen [pg/ml]	M:	40.46	116.40	213.75	92.21	78.58
	SD:	15.21	47.07	59.10	31.90	22.58
LH [mlU/ml]	M:	3.46	3.74	19.85	5.72	2.10
	SD:	1.05	1.32	8.85	1.67	0.95

venous blood was withdrawn, in order to determine the serum levels of hormones. Levels of the luteinizing hormone (LH) and 17β-estradiol (estrogen) were established in cooperation with the Department of Obstetrics and Gynecology, University of Erlangen-Nürnberg (radioimmunoassays provided by Serono, Italy: LH-MAIA Clone®; Estradiol MAIA®). A gynecologist determined the time of ovulation. He was not informed about changes in olfactory sensitivity.

SPSS-PC+ programs were used for statistical analyses. Since measurements were not obtained day by day, i.e. were not equally distributed in time, data were collected in five phases and mean values were calculated for each phase (see Doty et al.[9]). The time from the first day of the menstrual cycle (menstruation) to the day before ovulation was divided into two phases of equal length (phases 1 and 2). Phase 3 encompassed the day before and the day of ovulation. The time from the first day after ovulation to the last day of the menstrual cycle was again divided into two phases (phases 4 and 5). Data were evaluated in the following manner:

a) Data were submitted to analysis of variance (MANOVA, repeated measurement design, with 'phase' as 'within-subject-factor'; df 80/4). The prerequisites of the statistical model were tested with Mauchley's test of sphericity. In addition, the 5 phases of the cycle were submitted to trend tests (linear and quadratic trend) in order to analyze the type of function which best described the relation between changes in the measurements and time.

b) Correlations between serum levels of hormones and mood state and all other recorded parameters were separately calculated for each subject. After Z-transformation, the mean values of all correlation factors were calculated (n = 14).

Results

For mean values and standard deviations see the table.

Detection thresholds. Statistical analyses revealed no significant differences in detection thresholds among the 5 phases. Mean values revealed a slight decrease in detection thresholds for androstenone during phase 3 – that is, at the time of ovulation. However, this finding was considerably influenced by data obtained in only one subject. Her olfactory sensitivity substantially increased 1 day before ovulation. In all other subjects the detection thresholds did not change to any extent. In some subjects, the thresholds even tended to be slightly higher at the time of ovulation.

Hedonic ratings. Analysis of variance revealed significant differences in the hedonic ratings exclusively for androstenone ($F = 3.47$; $p < 0.05$). Androstenone was most pleasantly perceived at the time of ovulation. Trend analysis revealed a quadratic relationship between androstenone and the phase of the cycle ($t = 2.71$, $p < 0.05$).

In contrast, the hedonic ratings of nicotine and phenylethyl alcohol did not change in the course of the cycle.

Mood states. Mood scores showed a maximum at the time of ovulation. However, MANOVA ('phase' as within-subject-factor) revealed no significant differences among the 5 phases. There were no statistically significant correlations with other parameters.

Serum levels of LH and estrogen. The serum levels of estrogens and LH, measured in all 5 phases, concurred with data already known from the literature[10]. Again, there were no statistically significant correlations with other parameters.

Discussion

The present work yielded interesting findings with regard to the changes in hedonic ratings of androstenone during the menstrual cycle. At ovulation it was more pleasantly perceived than at any other time.

Since these changes in the hedonic ratings of androstenone coincide with the maximum of mood scores at ovulation[11,12], one could be tempted to assume that at this time, odorants are generally perceived to be more pleasant than at any other time. However, the absence of a significant correlation between the mood scores and the hedonic ratings of androstenone, as well as the non-appearance of changes in the hedonic ratings of other odorants (phenylethyl alcohol and nicotine) strongly argue against this assumption. Instead, it can be assumed that a selective change in olfactory perception occurs at the time of ovulation which is reflected in the changed hedonic ratings of androstenone.

Androstenone is a steroid derived from male sex hormones[13]. Men secrete androstenone to a larger extent than women[14,15]. In animals, the influence of odors in reproductive physiology is well known, and has been shown in several mammals[16–18]. Given the results obtained in experimental animals, it is conceivable that the smell of androstenone might also modify human behavior in a manner comparable to that of pheromones[19–23]. Provided that the smell of androstenone has been perceived at all[8], the change in the perception of this odorant at ovulation might favorably influence reproduction. This hypothesis is supported by findings of Adams et al.[24], who were able to demonstrate an increase in female-initiated sexual activity at ovulation.

This study revealed that the olfactory detection thresholds of healthy women do not change to any extent. However, in some subjects a substantial reduction in olfactory detection thresholds does indeed occur (see Le Magnen[25]). The reason for this occasional reduction is as yet unknown (anecdotal annotation: the subject in whom lowered thresholds were found at ovulation became pregnant two months after termination of the experiments).

Since considerable variations in detection threshold data have been observed[26], it is conceivable that the methods employed in this, as well as in all other previous studies, are not precise enough to accurately measure possible small changes in olfactory thresholds during the menstrual cycle. It is possible that other methods, such as the signal-detection-paradigm successfully employed by Doty et al.[6], might be better suited to registering marginal changes in olfactory sensitivity[27].

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1 Le Magnen, M. J., Arch. scient. Physiol. *6* (1952) 125.
2 Köster, E. P., Int. Rhin. (Leiden) *3* (1965) 57.
3 Vierling, J. S., and Rock, J., J. appl. Physiol. *22* (1967) 311.
4 Amoore, J. E., Popplewell, J. R., and Whissel-Buechy, D., J. chem. Ecol. *1* (1975) 291.
5 Kloek, J., Folia psychiat. neurol. neurochir. Neerl. *64* (1961) 309.
6 Doty, R. L., Snyder, P. J., Huggins, G. R., and Lowry, L. D., J. comp. Physiol. Psychol. *95* (1981) 45.
7 Hobi, V., Basler Befindlichkeitsbogen. Beltz Test Gesellschaft, Weinheim 1985.
8 Wysocki, C. J., and Beauchamp, G. K., Proc. natl Acad. Sci. USA *81* (1984) 4899.
9 Doty, R. L., Ford, M., Preti, G., and Huggins, G. R., Science *190* (1975) 1316.
10 Bardin, C. W., and Thau, R. B., in: Physiological Basis of Medical Practice, 11th edn, p. 921. Ed. J. B. West. Williams and Wilkins, Baltimore 1985.
11 Bäckström, T., Sanders, D., Leask, R., Davidson, D., Warner, P., and Bancroft, J., Psychosom. Med. *45* (1983) 503.
12 Warner, P., and Bancroft, J., J. psychosom. Res. *32* (1988) 417.
13 Brooksbank, B. W. L., Brown, R., and Gustafsson, J. A., Experientia *30* (1974) 864.
14 Claus, R., and Alsing, W., J. Endocr. *68* (1976) 483.
15 Gower, D. B., Bird, S., Sharma, P., and House, F. R., Experientia *41* (1985) 1134.
16 Hess, J. P., in: Comparative Ecology and Behaviour of Primates, p. 505. Eds R. P. Michael and J. R. Crooks. Academic Press, New York 1973.
17 Izard, M. K., in: Pheromones and Reproduction in Mammals, p. 253. Ed. J. G. Vandenbergh. Academic Press, New York 1983.
18 McClintock, M. K., in: Pheromones and Reproduction in Mammals, p. 113. Ed. J. G. Vandenbergh. Academic Press, New York 1983.
19 Russell, M. J., Switz, G. M., and Thompson, K., Pharmac. Biochem. Behav. *13* (1980) 737.
20 Benton, D., Biol. Psychol. *15* (1982) 249.
21 Filsinger, E. F., Braun, J. J., Monte, W. C., and Linder, D. E., J. comp. Psychol. *98* (1984) 219.
22 Cutler, W. B., Preti, G., Krieger, A., Huggins, G. R., Ramon Garcia, C., and Lawley, H. J., Horm. Behav. *20* (1986) 463.
23 Gustavson, A. R., Dawson, M. E., and Bonett, D. G., J. comp. Psychol. *101* (1987) 210.
24 Adams, D. B., Ross Gold, A., and Burt, A. D., N. Engl. J. Med. *299* (1978) 1145.
25 Le Magnen, M. J., C. r. Acad. Sci. *230* (1950) 1103.
26 Stevens, J. C., Cain, W. S., and Burke, R. J., Chem. Senses *14* (1989) 753.
27 Parlee, M. B., Psychol. Bull. *93* (1983) 539.

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