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Olfactometric and rhinomanometric outcomes in post-menopausal women treated with hormone therapy: a prospective study

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BACKGROUND: The aim of this prospective study was to evaluate the effects of hormone therapy (HT) on olfactory sensitivity in post-menopausal women. METHODS: Forty-six naturally post-menopausal women underwent rhinomanometric and olfactometric measurements to compare nasal airflow resistance values and olfactometric thresholds during the eighth month of HT treatment with baseline levels prior to starting HT. Eighteen women used an oral HT regimen, and twenty-eight women used transdermal patch HT. RESULTS: Rhinomanometric values during HT were statistically different from those at baseline ($P < 0.001$). Olfactometric threshold data indicated a higher sensitivity during the HT treatment than at baseline ($P < 0.001$). Finally, no statistically significant difference was observed among women using oral or patch HT administration on rhinomanometric and olfactometric values. CONCLUSIONS: Our study demonstrates that 8 months of treatment with estrogen and progestogens in HT preparations has an effect on nasal airflow resistance and the olfactory thresholds to odours. We believe that estrogens could influence neuronal plasticity, and the neuronal conduction time into the olfactory system. Our findings confirm that gonadal steroids such as estrogen have an influence on non-genital targets; this relationship might have a beneficial impact on sensorineural communication and emotional behaviour.

Key words: hormone therapy (HT)/olfactometric threshold/post-menopause/rhinomanometry

Introduction

Menopause is associated with many alterations of functional and organic well-being. The physiological changes that occur during menopausal transition and the post-menopausal period are a result of decreasing ovarian function with a reduction in ovarian hormones, mainly estrogens (Hammond, 1996). During the menopausal transition, the serum levels of estrogens decrease and subsequently remain low (Burger, 1999).

Hormone therapy (HT) is widely used to treat different aspects of the menopausal syndrome such as vaginal dystrophy, hot flushes and sweating, and to protect post-menopausal women against bone loss, cardiovascular diseases and Alzheimer's disease. Besides well-known genital targets, there are different non-genital targets for gonadal hormones (Caruso *et al.*, 2000a).

Olfactory function is markedly altered in old age and in a number of age-related diseases. The deficits appear to be rather general and are detectable by several types of olfactory tests (Doty, 1989). Considerable interindividual variability exists, however, and the physiological bases of these changes are not clear. In many healthy elderly persons, loss of smell appears to occur as a result of one or more cause, including viral insult, trauma, upper respiratory tract infection, sinonasal disease, congenital anosmia, idiopathic causes, cumulated exposure to toxic fumes and calcification of the cribriform plate (Kern *et al.*, 2004; Temmel *et al.*, 2002). Although there has been research both on sex differences in the ability to identify odors (Doty *et al.*, 1985) and on the biology of olfaction (Farbman, 1992; Ship *et al.*, 1996), the direct role of gonadal hormones on neurotrophic activities and sensory processing has not been studied. Only recently have studies conducted with pre-menopausal women addressed the effects of sexual steroids on olfaction in terms of both the physiological and biological aspects. In fact, the airflow and transnasal pressure during spontaneous respiration, as well as the olfactory thresholds to odours, seem to depend on the variation of the ovarian steroids that occur during the menstrual cycle (Grillo *et al.*, 2001). In women, rhinomanometry performed throughout the menstrual cycle showed higher airflow during the periovular phase than during the follicular

and luteal phases, and contraceptive use produces rhinomanometric and olfactometric values similar to those of the luteal phase (Caruso *et al.*, 2001).

The nasal respiratory epithelium has receptors for ovarian hormones, and estrogen plays an important role due to its trophism. Studies conducted during the menstrual cycle showed that the nasal mucosa became hyperactive to histamine in connection with ovulation when the blood estrogen level reached its peak (Haeggstrom *et al.*, 2000).

The ageing process has significant effects on sensory systems, and with respect to olfaction a decline in the ability to detect and discriminate odours has been reported. A loss of olfactory cells with increasing age was also evident in studies of both humans and laboratory animals (Ship *et al.*, 1996). In the young adult, the transition line between olfactory and respiratory epithelium is straight and distinct. In the septum of ageing mice, the transition line between olfactory epithelium and respiratory epithelium becomes interdigitated and tortuous (Rosli *et al.*, 1999). A similar irregular olfactory epithelium and respiratory epithelium boundary in ageing, and possibly during disease, has also been reported in humans (Naessen, 1971). This feature may reflect respiratory epithelium compensation for neuroepithelial losses (Nakashima *et al.*, 1984). Our study confirmed that the nasal respiratory epithelium is an estrogen target, just as vaginal cells are. The HT activities in the nasal respiratory epithelium could depend on the type of hormone regimen used (Caruso *et al.*, 2003).

The literature reports that ageing is accompanied by olfactory loss and hyposmia/anosmia, which is also a feature of several neurodegenerative disorders (Kovacs, 2004). The olfactory loss may cause loss in quality of life (QoL) due to food-related problems. This loss in QoL seemed to be of greater importance in younger than in older people, and women seem to be affected more strongly than men (Temmel *et al.*, 2002).

The pathology of the olfactory mucosa is poorly understood; however, most cases of hyposmia and anosmia appear to be associated with a decline in the number of functioning mature olfactory sensory neurons. A common pathway may mediate olfactory sensory neuron cell death from a diverse set of pathological insults including ageing, trauma and sinusitis (Kern *et al.*, 2004). On the other hand, data from the literature are insufficient in showing the role of steroids on the olfactory system. Consequently, due to this lack of knowledge, we wanted to study, in a prospective trial, the airflow and the olfactory threshold changes before and after starting HT in post-menopausal women, excluding subjects with olfactory pathologies that could have influenced the physiology of smell.

Materials and methods

The study was performed at the Menopausal Service of the Department of Microbiological Science and Gynecological Science and at the Department of Otorhinolaryngology, School of Medicine, University of Catania, Italy. All subjects gave their written informed consent before participation in the study, which was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board of the research committees of both the Departments approved the study. The study was not advertised and no remuneration was offered. The study was conducted as a prospective study.

Subjects

Although there are controversies regarding the use of HT in symptomatic or asymptomatic post-menopausal women (LeBlanc *et al.*, 2001; Maki and Hogervorst, 2003), we enrolled symptomatic subjects, requiring HT treatment for climacteric symptoms. Seventy-three healthy women were admitted in the longitudinal study, ranging in age from 48 to 55 years (mean age 52.5 $\pm$ 3.3), who had been naturally menopausal for between 8 months and 5 years, and who were experiencing moderate to severe menopausal symptoms, such as hot flushes, night sweating, sleep problems, vaginal dryness and tiredness, (Kupperman index $\geq$15, with $\geq$1 hot flushes with sweating per day). None was using any HT. The plasma FSH levels were $>$40 IU/ml, and the serum estradiol levels were $<$30 pg/ml. Hormone concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Roche, Monza, Italy). The body mass index (BMI) of each woman was within the normal range (23.7 $\pm$ 1.6 kg/m^2). Inclusion criteria required a normal gynaecological history and examination, normal mammography result within 1 year, and no major abnormality of serum glucose, lipids or liver enzymes.

Before women were admitted to the study, a general medical and gynaecological examination was performed. Any significant otorhinolaryngological pathologies or medical problems such as extragenital hormonal diseases, current tobacco use or abuse of alcohol, and use of drugs excluded subjects from participation; any symptomatic treatment for climacteric symptoms, tranquillizers and antidepressant drugs was prohibited.

Clinical testing

Each woman was referred to the Olfactology Department for evaluation of both objective and instrumental aspects. All women underwent ear, nose and throat checks to look for inflammation of the upper airways. Screening tests using endoscopy, rhinomanometry and olfactometry were carried out.

Endoscopy was performed using a Pentax FNL 10S (Pentax, Asahi Optical, Osaka, Japan) with a television camera and an SWHS AG:4700 VHS Panasonic recorder (Matsushita Electric Industrial Co., Osaka, Japan). This permitted the visualization of the nasal cavity and the rhinopharynx without local anaesthetic, and the recording of the pictures obtained. During the pre-screening examination, by means of rhinoscopy, five women with atrophic lesions of the nasal mucosa, six subjects affected by polyps and two affected by deviated septum were excluded from the study. Therefore, 60 subjects were enrolled in the study.

Rhinomanometry is defined as the simultaneous measurement of nasal airflow resistance (R) before and after nasal decongestion during spontaneous respiration. This resistance can be expressed by the formula R = P/V, where P is the transnasal pressure and V is the volume of airflow. We used a Rhinospir 164 (Sibelmed, Barcelona, Spain) for this test. To test the olfactory threshold, we used the Fortunato-Niccolini olfactometer (Fortunato-Niccolini, Catania, Italy), with five different odour substances. To evaluate the sensitivity differences, rhinomanometric and olfactometric surveys were carried out at baseline and during the eighth month of HT treatment. Rhinomanometry was performed on each woman during both inhalation and exhalation, with transnasal pressure ranging between 300 and $-$300 mmHg, and with airflow ranging between 800 and $-$800 cm^3/s.

The substances used to test olfactory sharpness with the Fortunato-Niccolini olfactometer were among the best known monomolecular substances most suitable for a complete investigation: anise and musk-ketone with an exclusive olfactory component; pyridine, which, apart from the olfactory effect, adds to the stimulation of taste and trigeminal terminations; citral with an exclusive olfactory and trigeminal effect; and ammonia with a prevalent trigeminal component (Fortunato *et al.*, 1972; Murphy and Cain, 1980; Lawless and Zwillenberg, 1983; Keverne *et al.*, 1986). Each substance was used in the liquid state. Anise, musk-ketone and citral were pure essences; pyridine was a pure liquid substance; and ammonia was a liquid substance diluted to 22% in distilled water (Pharmacia, Milan, Italy). We considered the olfactory coefficient of each substance, which is the quantity of odorous air sufficient to reach a level of identification in ≤ 5 s: 2 ml for anise, musk-ketone and citral; 0.5 ml for ammonia; and 1 ml for pyridine. Subjects who do not perceive any substance in the range of its specific olfactory coefficient in >5 s are usually defined as being affected by hyposmia/anosmia. The Fortunato-Niccolini olfactometer contains six 500 ml glass bottles. We used five bottles, each of them containing 30 ml of a single substance. Each bottle was hermetically sealed by a rubber cap through which two tubes were passed, one connected to a syringe specially graduated in ml, the other to a connector leading to the nostrils. The graduated syringe was actuated by a physician, this increased the pressure inside the bottle forcing the same quantity of odorous air into the tube connected to the nostril, with an increase of 0.2 ml per test (0.2, 0.4, 0.6, etc.), up to its olfactory coefficient. The olfactometric test was carried out on all patients sitting in front of the terminal fork, which conducted the odours simultaneously to both the right and the left nasal vestibules; each woman was blinded to the test substance and dose (see the drawing of the olfactometer in Caruso *et al.*, 2001). Each odour was delivered to the nasal mucosa during normal inspiration. The test was repeated for each of the odorous substances, starting with 0.2 ml, until the patient perceived the odour. The threshold perception was given by the smallest volume of air able to make the subject perceive the odour. There was a wash-out period of 10 min between test doses.

We excluded smokers from our study, and requested that each woman should not start smoking, nor smoke an occasional cigarette, and not stay in environments with smokers for a least 1 day before the experiments started. Moreover, each woman was asked to avoid strong olfactory stimuli and not to use perfume for a similar time. The duration of one measuring session was ~ 1 h.

Finally, measurements of plasma estradiol levels were performed at baseline and during the eighth month of HT treatment.

Hormone therapy

After clinical testing, HT was prescribed to each woman. Of the 60 women, 28 were prescribed a continuous combined daily oral dose tablet of conjugated estrogens 0.625 mg and medroxyprogesterone acetate (MPA) 5 mg (Premelle C, Wyeth, Aprilia-Latina, Italy). Thirty-two women started a sequential continuous HT, with a once a week transdermal estadiol patch regimen (Climara 50, Schering, Milan, Italy), plus nomegestrole acetate 5 mg (Lutenyl, Theramex, Milan, Italy) used orally for 12 days per month. During the eighth month of HT, rhinomanometric and olfactometric surveys were performed. In order to standardize the methodology, women on the patch HT underwent testing during progestin intake.

Statistics

The intention to treat population was based on the efficacy and safety of HT used for each woman, with the last observation carried forward for patients who prematurely discontinued treatment.

Assuming an SD of 1 and a mean difference of 0.5 between before and after HT use olfactory values at $P = 0.05$, the sample size calculation indicated that 60 subjects would be the minimum number required for the study to have 80% power.

The rhinomanometric values obtained at baseline were compared with those obtained after HT intake using the Wilcoxon signed rank test. Each subject produced eight values, four from the inhalation and four from the exhalation section. Consequently, we obtained 240 values from each section at baseline (total 480), and 184 values at the eighth month of treatment (total 368). Paired data *t*-test was used to compare olfactometric values of each odorous substance obtained at baseline with the HT values. Finally, the two-sided *t*-test for independent samples was used to compare the effects of the two means of HT administration on both rhinomanometric and olfactometric aspects. All reported values are given as means ± SD. The differences were considered statistically significant for $P \leq 0.05$. Each statistical analysis was carried out using a software package for Windows 95 (Glantz, 1997).

Results

After excluding 13 women from the study for nasal pathologies (see clinical testing section), the sample consisted of 60 women 48–54 years of age, of which six (10%) discontinued treatment during the second month of treatment (four in the oral and two in the transdermal treatment); two (3.3%) withdrew during the first month of HT treatment for adverse events due to local intolerance to the patch, and six (10%) during the first 3 months of treatment because of lack of efficacy of the drug (in the oral regimen). For these, we only obtained the baseline testing, and thus they were excluded from any analyses. Therefore, 46 subjects completed the study, 18 using the oral HT regimen, and 28 using

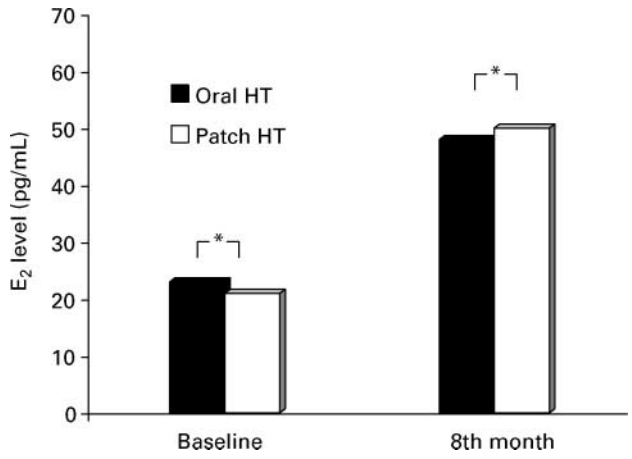

Figure 1. Plasma estradiol levels at baseline and during the eighth month of oral and patch HT treatment. Women taking HT by both modalities showed improved estradiol values with respect to those at baseline ($P < 0.001$). Differences were not statistically significant between groups. *P not significant. Data are expressed as mean ± SD.

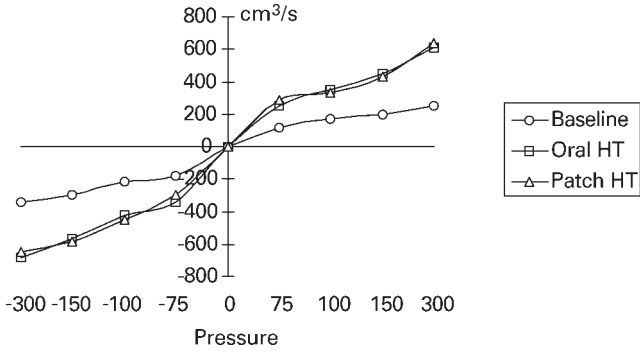

Figure 2. Inhalation (on the right) and exhalation (on the left) rhinomanometric values obtained at baseline and during the eighth month of HT usage.

transdermal HT. Finally, 40 out of 46 (86.9%) completed each olfactometric test, and the analyses are based upon the intention to treat population.

Figure 1 shows the levels of estradiol at baseline and at the eighth month of HT treatment. Both groups of women treated with patch and oral HT had an improvement in plasma estradiol levels with respect to the baseline ($P < 0.05$) of 50 ± 3.1 and 48 ± 3.4, respectively. There were no statistically significant differences between them.

Figure 2 shows both the inhalation and exhalation rhinomanometric values obtained at baseline and during the eighth month of HT use. At baseline and during HT use, the mean transnasal pressure with respect to the airflow value during inhalation and the exhalation time of the right nasal cavity was similar to that of the left nasal cavity (P = NS). Table I shows the Wilcoxon signed rank test statistical comparisons of inhalation and exhalation rhinomanometric values obtained during the HT intake with those at baseline and with respect to the modality of HT use. The rhinomanometric values during HT use were statistically different from those at baseline ($P < 0.001$). No statistically significant difference was observed among women using oral or patch HT on rhinomanometric values. Table II shows the olfactometric thresholds at baseline and during HT use. Each value is expressed as the mean ± SD ml of air able to make the subjects perceive the odour. Olfactometric threshold data indicated a higher sensitivity during the HT treatment than at baseline for each substance (Table III). Finally, no statistically significant difference was observed among women using oral and transdermal HT administration on olfactometric surveys for musk-ketone [$t = 0.20$; confidence interval (CI) = -0.0891 to 0.1098], citral ($t = -1.35$; CI = -0.1246 to 0.0245), anise ($t = 0.84$; CI = -0.041 to 0.1019), pyridine ($t = -0.65$; CI = -0.1221 to 0.0621) and ammonia ($t = 0.90$; CI = -0.0244 to 0.0644) with 44 degrees of freedom.

Table II. Olfactometric thresholds during pre treatment and during HT intake

Odorous substances	Baseline ($n = 60$)	HT ($n = 46$)
Mush-ketone	2.21 ± 0.16[a]	1.90 ± 0.14
Citral	2.17 ± 0.12	1.92 ± 0.16
Anise	2.16 ± 0.12	2.06 ± 0.11
Ammonia	0.60 ± 0.07	0.47 ± 0.08
Pyridine	1.16 ± 0.17	0.96 ± 0.23

[a]Values are mean ± SD ml of air.

Table III. Statistical comparison analysis of the HT intake versus the values at baseline during the olfactometric test

Odorous substances	*t*	95% CI	*P*
Mush-ketone	16.11	0.2754–0.354	<0.001
Citral	12.75	0.2061–0.2833	<0.001
Anise	9.14	0.07964–0.1246	<0.001
Ammonia	11.43	0.1069–0.1526	<0.001
Pyridine	7.53	0.145–0.2507	<0.001

Degrees of freedom between groups = 1, within groups = 45.
t = paired data *t*-test; CI = confidence interval.

Discussion

The subjective and objective complaints and the organic and metabolic damage of post-menopausal women depend on estrogen deficiency syndrome (World Health Organization, 1996).

Estrogen deficiency affects some topographically remote organs and is manifested by an apparently clinically heterogeneous symptomatology. Clinical observations strongly suggest that changes in gonadal function modify auditory, olfactory and taste thresholds (Velle, 1987). Estrogen deficiency produces multiple extragenital effects of gonadal steroids. Post-menopausal women treated with HT may have changes in the cytological aspect of the larynx (Caruso *et al.*, 2000a) and of the nasal respiratory epithelium (Caruso *et al.*, 2003), and in the auditory brainstem response (Caruso *et al.*, 2000b).

Table I. Statistical comparison analysis by Wilcoxon signed rank test of the HT intake values obtained during the inhalation and exhalation rhinomanometric test

Modality of HT	Inhalation				Exhalation			
	W	τ_i	Z_w	*P*	W	τ_i	Z_w	*P*
Oral HT	−1053	47	5.57	<0.001	1128	47	5.96	<0.001
Patch HT	−1102	43	5.19	<0.001	1097	45	5.72	<0.001

W = signed rank test; τ_i = overriding rank number; Z_w = statistical Wilcoxon test.

Our study investigated the changes in the rhinomanometric and olfactory thresholds of naturally post-menopausal women treated with HT. In contrast to other studies that were unable to detect any differences between post-menopausal women receiving HT and those not receiving HT (Hughes *et al.*, 2002), or treated with progestins alone (Albertazzi, 2002), our study based on a methodology including both rhinomanometry and olfactometry demonstrated that 8 months of estrogen and progestogens in HT preparations has an effect on airflow and transnasal pressure and the olfactory threshold. This effect correlates with the rise in serum estrogen levels seen after HT treatment. In fact, our results suggest the existence of a better olfactory threshold that was sensitive to a rise in estradiol during the HT, seen by modifications in the plasma estradiol levels. This could be due to an increased airflow with HT, indirectly causing a decrease in olfactory threshold. We noted this effect in different phases of the menstrual cycle (Grillo *et al.*, 2001) and in women taking oral contraceptive preparations (Caruso *et al.*, 2001). However, we hypothesize that HT could also act directly on neuronal transmission; this will be the basis of a future study, using olfactory evoked potentials in order to verify this hypothesis.

In odour recognition tests, it has been demonstrated that an odour will not be forgotten for at least 30 s (Engen *et al.*, 1973), 3 days (Baker and Weaver, 1983) or even 1 year (Engel and Ross, 1973). These data suggest a very long-lasting, possibly permanent memory for olfaction traces. In fact, the amygdala can be activated by single odours. Because the amygdala has a role in influencing emotional behaviour, it is highly probable that there is an immediate activation of this structure during the passive perception of odors, and that this underlies the common experience that olfactory stimuli produce immediate recall of the emotional reaction related to the source of smell (Savic, 2001). The same mechanism may also explain the long duration of odour memories. The substances that we used to test olfactory sharpness were among the best known monomolecular substances most suitable for a complete investigation, and they are commonly found in nature. Consequently, subjects might have been familiar with each of them. In studies on the nature and duration of adaptation, long-term odour exposure produces an odour-specific reduction in sensitivity and perceived intensity compared with pre-exposure baseline (Dalton and Wysocki, 1996). On the contrary, we used a specific and unique substance for each test. In order to minimize any possibility of learning, we adopted the following: (i) each subject underwent the second olfactometric testing 8 months after the first; and (ii) each woman was blinded to the test substances and dose, and each substance was used just once during each session. Finally, we think that it could be useful to study the olfactory threshold of our subjects during an interruption of HT. This procedure might permit the definition of any eventual level of learning and /or experience with the olfactory test.

Our findings confirm that gonadal steroids such as estrogen have an influence on non-genital targets; this relationship might have a beneficial impact on sensorineural communication and emotional behaviour.

Although the mechanisms by which estrogen affects these olfactory changes remain to be established, future studies could indicate a role for HT in the treatment of sensory delay. In fact, our trial could be important for research into changes in sensory communication.

Finally, estrogen deficiency can be manifested by clinically heterogeneous symptoms. The Kallman syndrome, a disorder characterized by hypogonadotrophic hypogonadism and anosmia, could help in understanding the interaction between estrogen and olfaction. The syndrome indicates that smell is important in sexual development, because LH-releasing cells of the hypothalamus arise from progenitor cells in the olfactory placode (Hu *et al.*, 2003).

Available evidence thus suggests that HT may be a prevention, and possibly treatment, of the alteration of olfactory sensitivity that could occur in post-menopausal women. The common origin of all pathological conditions arising from estrogen deficiency calls for interdisciplinary collaboration in the new discipline of climacteric medicine.

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