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EFFECT OF PUTATIVE PHEROMONES ON THE ELECTRICAL ACTIVITY OF THE HUMAN VOMERONASAL ORGAN AND OLFACTORY EPITHELIUM

L. MONTI-BLOCH and B. I. GROSSER
Department of Psychiatry, School of Medicine, University of Utah, Research Park, W.I.N., Salt Lake City, UT 84108, U.S.A.

Summary—The summated receptor potential was recorded from the vomeronasal organ (VNO) and olfactory epithelium (OE) of 49 human subjects of both sexes (18 to 55 years old) using surface non-polarizable silver–silver chloride electrodes. 15–25 pg of human putative pheromones, clove oil and a diluent were administered to the VNO or the OE in 0.3–1 s pulses from a 0.05 mm dia cannula connected to a multichannel delivery system. Local stimulation of the VNO produces negative potentials of 1.8–11.6 mV showing adaptation. Responses are not obtained when the recording electrode is placed in the nasal respiratory mucosa. Pheromone ER-830 significantly stimulates the male VNO ($P < 0.01$; $n = 20$), while ER-670 produces a significant effect on female subjects ($P < 0.001$; $n = 20$). The other pheromones tested do not show significantly different effects in both male and female ($P > 0.1$). Similar quantities of odorant or diluent produce an insignificant effect on the VNO. Stimulation of the OE with clove oil produces depolarization of 12.3 ± 3.9 mV, while pheromones do not show a significant effect. Our results show that the VNO is a functional organ in adult humans having receptor sites for human putative pheromones.

INTRODUCTION

Chemical communication by pheromones is known to be essential for reproduction in many invertebrates and vertebrates, and has been the subject of extensive investigations. It is generally accepted that all terrestrial vertebrates possess a vomeronasal (VN) system that plays an essential role in the pheromone detection and in the integration of such sensory information with reproductive behavior mediated by the anterior hypothalamus and limbic system [1–9]. In many mammals, stimulation of the VN system activates neuroendocrine reflexes governing the release of gonadotrophins and the induction of sexual behavior. For example, in several species male detection of species-specific pheromones present in the urine and vaginal secretions of the female provides essential cues for the detection of estrus [4, 6, 9, 11–14]. Mating fails to occur if the VN system is experimentally compromised [3, 9].

The VN detection system is situated within bilateral tubular structures, the vomeronasal organ (VNO) or Jacobson's organ [3, 9, 15–17]. The lumen of this blind cavity is lined with sensory epithelial elements which constitute a distinctive subset of olfactory receptors. In mammals, the VNO is located beneath the respiratory mucosa of the nasal septum and is independent of the olfactory epithelium (OE). In most species communication with the nasal cavity occurs by means of an aperture near the nares, but in a few animals it opens via a duct into the oral cavity. The sensory elements of the organ consist of elongated neuroepithelial cells provided with microvilli. They differ from olfactory receptors in that they lack cilia [8, 17–19]. Tracer studies reveal that the central process of VNO receptors project to the glomerular layer of the accessory olfactory bulb (AOB) via the VN nerve [1]. In many species, receptor axons are accompanied by nerve fibers of an accessory cranial nerve, the nerve terminalis, arising from bipolar neurons situated in a submucosal plexus which provides direct connections between the VNO and the medial preoptic nucleus of the hypothalamus [1, 9, 20]. The nerve cell bodies and axons of the nervus terminalis are immunoreactive for LHRH and provide a critical monosynaptic linkage between the VNO and the brain [10, 21–24].

In some mammals, non-volatile pheromone stimuli reach the lumen of the VNO by a

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pumping mechanism involving the autonomic nervous system [25–27] and stimulation of the receptors induces a local depolarization similar to the receptor potentials in other chemoreceptor organs [28–34]. Following stimulation of the VNO, synchronous discharges occur not only in the VN nerve, but have also been recorded in the AOB neurons, neurons of the medial preoptic area of the hypothalamus, and the amygdala [3, 35].

The human VN system, in contrast to other mammals, has received relatively little attention and there is considerable disagreement as to its functional status. Some authors believe that the adult human VN system is a vestigial structure, having functional significance only during the early stages of life, possibly with regard to maternal recognition by the infant [3, 9, 22]. Electronmicroscopic studies of the human VNO have recently shown that there are two potential receptor elements in the epithelium lining of this organ [1] and the otorhinolaryngological study of Garcia-Velasco and Mondragon [36] identified the VN pit in 850 out of 1000 human adults of both sexes. Other authors have also reported its presence in the adult [16, 19, 22, 23, 37–39].

We have addressed our neurophysiological investigation to two issues in view of preliminary findings which indicated a functional VN system in the human: (a) whether the adult human VNO responds to specific exogenous chemosensory signals; and (b) whether the electrophysiological results of such stimulation resemble those of other mammals.

A system has been devised to stimulate the human VNO and to record its electrical activity in unanesthetized subjects [40, 41]. Since the olfactory mucosa has been proposed by others as the site of action of pheromones [5, 9, 42], we have also studied the effects of a variety of potential pheromonal substances on the changes in electrical activity of olfactory receptors, cortical-evoked potentials, the galvanic skin response, cutaneous temperature and peripheral arterial pulse. The following is a description of our work.

METHODS

The study was performed in 49 clinically normal (screened) volunteers ranging from 18 to 55 years of age, without the use of anesthetics. Female subjects were excluded if they were pregnant or if they were not using contraceptive techniques.

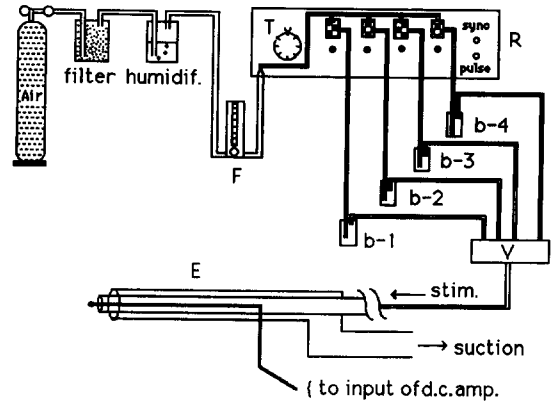

Fig. 1. Schematic diagram of experimental apparatus used to chemically stimulate the VNO. Compressed air was passed through an activated charcoal filter, humidified, the rate flow adjusted to 0.03 ml/s (F) and fed to a multi-channeled delivery system (R). Odorants and pheromone dissolved in diluent, situated in chambers "b" were bubbled with air. Air pulses containing known dilutions of the test stimuli were created by an electronic switch controlling each channel at V. The output was fed to the stimulating cannula E and its outflow was continuously scavenged by the surrounding suction cannula. For details of the recording electrode, see the text.

Electro-vomeronasogram (EVG)

A schematic diagram of the stimulating system is illustrated in Fig. 1. The recording electrode consists of a 0.08 mm dia silver wire insulated with Teflon (AM Systems) whose tip has been melted in an oxygen–acetylene flame to form a bare 0.3 mm ball, the surface of which is then coated with silver chloride and covered with 6% agarose–saline.

The silver Teflon wire is positioned within a 10 cm Teflon catheter (o.d. = 1 mm) such that the chlorided ball protrudes approx. 1 mm beyond the opening of the catheter while the other end is connected to a d.c. preamplifier. The Teflon catheter is part of a multichannel delivery system involving pulses of air from chambers containing the pheromone in diluent. The catheter electrode is enclosed within a second Teflon tube with a dia = 2 mm. This is connected to an aspirator that provides for continuous suction of 0.3 ml/s and is positioned so its tip is outside the VNO but within the pit at the surface of the nasal septum. This concentric arrangement allows chemical stimulation to be localized to the VNO and avoids diffusion of substances to the neighboring olfactory area or into the respiratory system. Subjects used in these studies failed to report any sensation of pain as a consequence of EVG measurements.

Recording procedure

The recording was carried out in a quiet room with the supine subject lying on a bed with a small pillow under the neck to allow comfortable neck extension. Reference and ground electrodes also consist of chlorided silver discs (dia = 8 mm). The reference electrode is placed on the glabella, and the ground electrode is attached over the mastoid process using Redux Gel (Hewlett Packard) and adhesive hypo-allergenic discs. The VN pit is identified close to the intersection of the posterior edge of the septal cartilage and the nasal floor by opening the right nare using a nasal speculum.

The Teflon catheter containing the recording electrode is then gently introduced to the VNO for a distance of 1–3 mm and held in place with surgical hypo-allergenic tape. The nasoscope is then withdrawn. During recording the subjects are asked to breathe through the mouth.

Electrical potentials from the d.c. low-noise amplifier are digitized (sampling rate = 100 kHz) (MacLab/4, World Precision Instruments Inc.) and stored utilizing an on-line computer (Macintosh SE 30, Apple). The peak-to-peak amplitude of the locally recorded electrical potentials is then measured and also their area integrated, while continuously monitored both on the computer screen and on a digital oscilloscope. Electrical or mechanical artifacts caused by movement of the subject, deep breathing, swallowing or blinking are then deleted.

Chemical stimuli

Test substances included: diluent and Clove oil (Sigma) as controls, and ER-670, ER-830, ER-700, ER-795 and ER-360 as test compounds.* Experimental samples of putative pheromones or controls were diluted in concentration of 15–25 pg and loaded in separate containers. Each container was part of a separate channel of our delivery system. Clean, humidified air at 34°C was continuously bubbled through each container. A chemical stimulus consisted of pulses of saturated air lasting 300, 500 or 1000 ms. Usually an interval of at least 5–10 min separated each series of air pulses. All the lines carrying the stimuli were Teflon, glass or stainless steel which were carefully cleaned for each subject.

*These putative pheromones were supplied by EROX Corporation

Electro-olfactogram (EOG)

Recordings from the olfactory mucosa employed the same stimulating and recording system as that used for the VNO [41]. The tip of the recording electrode is positioned in the lateral part of the medial nasal duct and slowly introduced until it reaches the olfactory mucosa. Adequate contact is signaled by local depolarization in response to odorous test stimuli which were the same as those used for stimulation of the VNO. As with EVG measurements, no pain was reported as a result of this procedure.

Galvanic skin response (GSR)

Recordings employed 8 mm dia silver electrodes in contact with the skin of the medial and annular fingers by means of a conductive gel interface. Signals were generated by 10 μA d.c. pulses which were continuously passed through one of the electrodes. Skin conductance changes were d.c. amplified and monitored on a computer screen.

Skin temperature (T)

A mini thermistor probe (Biopac Systems) attached to the skin of the right ear lobe permitted temperature changes to be continuously monitored on a computer screen.

Plethysmography (P)

The peripheral arterial pulse was monitored using a mini plethysmosgraph (Biopac Systems), attached to the index finger. The signal was d.c. amplified and continuously monitored on a computer screen.

Statistical analysis

The significance of the results was determined by either using paired t-tests or analysis of variance (ANOVA).

RESULTS

Characteristics of the human EVG

Air pulses containing picogram quantities (15–25 pg) of putative pheromones were applied to the VNO as pulses of 300 ms to 1 s duration. A negative potential was recorded in response to certain pheromone stimuli only when the active electrode was in contact with the sensory epithelium. Similar potentials of lesser amplitude were obtained when standard olfactory test stimuli such as clove oil, amyl-acetate or diluent were employed.

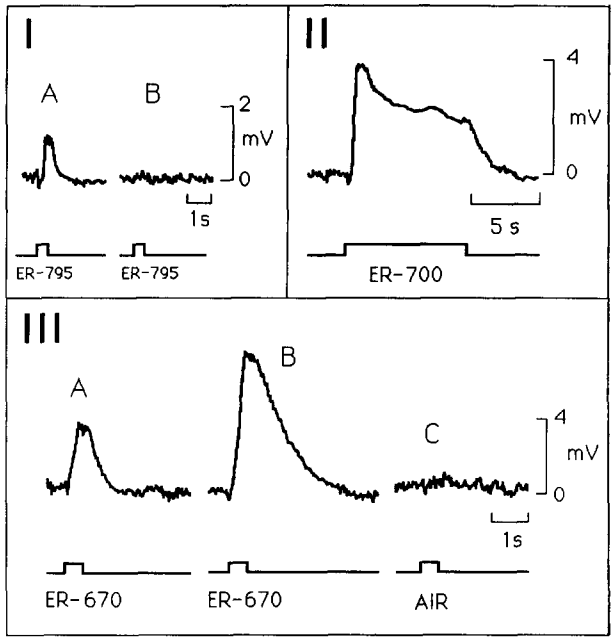

Fig. 2. Characteristics of the receptor potential recorded from the human VNO. I: a 500 ms air pulse (A) produced negative depolarization in a female subject; (B) note the absence of response when recording from neighboring respiratory mucosa. II: a prolonged (10 s) air pulse of ER-700 (25 pg) induced transient depolarization, followed by a plateau and slow decay during maintenance of the stimulus (adaptation). III, stimulation of a female VNO with 500 ms air pulses of ER-670: (A) a single air pulse of 15 pg produced a 4 mV negative potential; (B) 5 min later, 25 pg of ER-670 produced a larger amplitude and duration of the response; the rising and falling slopes are similar in A and B; in C there is an absence of response when an air pulse is delivered to the VNO.

As shown in Fig. 2(I-A), a typical negative potential was produced by local stimulation with 25 pg of putative pheromone ER-795 and was recorded from the VNO of a 31-year-old woman. Depolarization rises steeply reaching a peak at 2 mV and decaying to baseline with a slower slope. The long latency of the potential (200 ms after the application of the stimulus) is probably due, in large part, to the time required for arrival of the stimulus at the receptors. In order to minimize the delay we decreased the length of the stimulating cannula-electrode to 10 mm. Importantly, if the recording electrode is positioned in nearby respiratory mucosa, stimulation produces no effect. A response is also absent when the recording electrode is in contact with the VNO epithelium and stimuli are applied either to the OE or nasal respiratory mucosa by means of a "mobile" cannula. Thus, the VN negative potential is produced by specific chemosensory stimuli delivered to the sensory epithelium of the VNO.

The size of the potential recorded from the VNO is a function of stimulus strength. As shown in Fig. 2(III), the right VNO of a 42-year-old woman was stimulated with air pulses produced by different concentrations of pheromone ER-670. Trace A shows the response to a 500 ms air pulse of low concentration (15 pg). This stimulus produced a negative potential with a steep rising phase, followed by a slow decay toward the baseline. In trace B, a second air pulse containing a higher concentration (25 pg) was delivered which produced a larger response with a steeper rising phase. Also, the duration of the response was increased. In trace C, a pulse of pure air induced an insignificant change in potential.

The absence of a response to air shows that the potentials recorded in traces A and B are biological events produced by chemical stimulation of the receptors and are not due to artifacts induced by mechanical stimulation.

Evidence that the character of the rising phase of the negative potential is a function of qualitative differences between the putative pheromonal stimuli is provided in Fig. 3. Here, the concentration of pheromone was adjusted to 25 pg. Stimuli were presented to the VNO at 5 min intervals to allow full recovery between applications and all stimuli were delivered as 500 ms air pulses. A series of responses differing in amplitude but with a similar rising phase follows each stimulus, e.g. ER-670 which shows

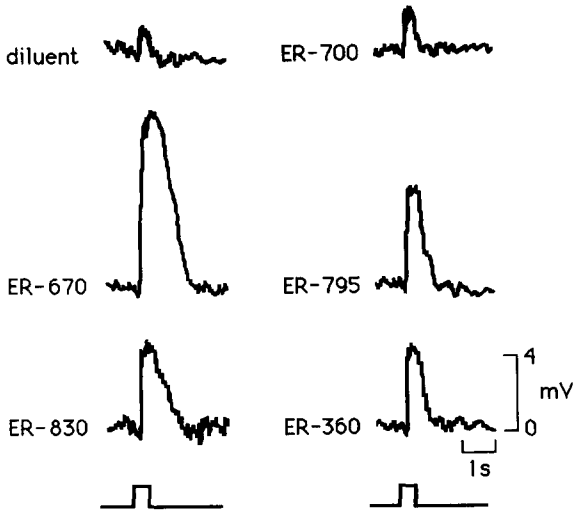

Fig. 3. Putative pheromones (25 pg) delivered in 500 ms air pulses at 5 min intervals to the VNO of a female subject produce receptor potentials of differing amplitude and characteristic decay. Note that ER-670 has the largest effect (9.3 mV) while the control diluent pattern has a weak effect (1.4 mV).

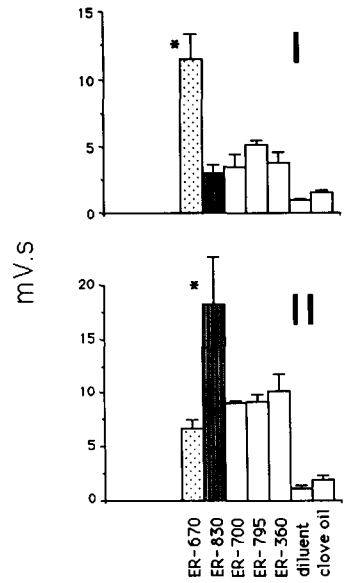

Fig. 4. Sexual differences in the response of the VNO to the same pheromones. Bars are the mean response in a population of females ($n = 20$) and males ($n = 20$). I: ER-670 produces a significant effect ($P < 0.001$) on female subjects. II: ER-830 significantly stimulates the male VNO ($P < 0.01$). ER-670 produces less effect in males and ER-830 is less potent in females. Other pheromones do not show significant differences in both populations ($P > 0.1$). In both cases the diluent and clove oil have a weaker effect, significantly different from that of pheromones ($P < 0.01$).

the largest amplitude, but the rising phase has similar short latency of onset and peak rise time to those of the diluent, or ER-700, which has smaller amplitudes. On the other hand, the recovery phase of these potentials recorded from the VNO epithelium has a characteristic signature in response to each stimulus.

Thus, while responses to ER-830 and ER-360 produce receptor potentials of similar amplitude ($\Delta V = 6.2 \pm 0.4$ mV), the effect of ER-830 requires longer to decay to the baseline (2.8 s) than that of ER-360 (2 s). This has led us to measure the area of the VNO potentials as the most appropriate way to characterize the response to each stimulus.

Long-lasting stimulus produces adaptation of the VNO receptors, a universal property of all receptor cells [30–34]. In the experiment of Fig. 2(II), the VNO was presented with a 10 s stimulus. Although the response shows an initial peak followed by a plateau that persists throughout the stimulus and ends with an exponential decay to baseline, when the stimulus is terminated, the plateau shows a progressive reduction in amplitude indicative of adaptation. VNO receptors also show adaptation to stimulation with repetitive air pulses of short duration. The response to 300 ms air pulses decreases in amplitude with successive stimuli. Therefore, the potentials recorded from the VNO epithelium have similar properties to the summated receptor potentials from depolarized groups of receptor cells in other organs [30, 32, 34]. Although intracellular recordings from individual receptor cells are needed, we consider potentials recorded from the VNO epithelium to be summated receptor potentials.

Effects of human pheromones on the EVG

For many of the putative pheromones tested we noticed that the receptor potential varied depending on the sex of the subject. Thus, we have conducted experiments to study their difference in the EVG of female and male subjects. Examples are shown in Fig. 4(I,II) and also in Table 1.

Recordings of the EVG were done in a population of 20 women (18 to 55 years old), divided in two groups: 17 fertile women in the preovulatory stage of their menstrual cycle; and 3

Table 1. Effect of putative human pheromones and controls

	Mean		SEM	
Substance	Males (M)	Females (F)	Males (M)	Females (F)
ER-670	6.6	11.45*	1.13	1.49
ER-830	18.2*	2.96	4.4	0.52
ER-700	9.05	3.47	0.15	0.74
ER-795	9.1	5.12	0.7	0.41
ER-360	10.1	3.79	1.55	0.79
Diluent	1.14	0.86	0.3	0.12
Clove oil	1.9	1.53	0.4	0.14

$n_F = 20$; $n_M = 20$.
*$P_M < 0.01$; $P_F < 0.001$.

menopausal subjects (see Table 1). Test odorant, diluent, and putative pheromones were diluted and applied to the VNO in 1 s air pulses at 5 min intervals. When questioned, the subjects responded smelling none of the common odors. This indicated that the odorant cannula was in fact delivering test stimuli to the VNO with no escape to the adjacent olfactory mucosa since air pulses of common test odors were always correctly identified when applied to the olfactory mucosa of the subjects.

Figure 4(I) shows the averaged response of the female subjects to a classic odorant (clove oil), to the diluent, and to five putative pheromones (ER-670, ER-830, ER-700, ER-795, ER-360). The profile of the response to each of the substances was similar in all subjects regardless of age and no significant differences were revealed by t-tests and ANOVA. For example, ER-670 produced a significant effect ($\bar{X} = 11.45$ mV·s; SEM = 4.17; $P < 0.001$) that was consistent in all individual cases. Other pheromones depolarized the VNO receptors to a lesser extent, with consistent mean response amplitudes from individual to individual. All pheromones produced larger responses than did the diluent alone or the olfactant clove oil ($P < 0.001$).

A similar experimental protocol was followed with 20 male subjects whose age ranged from 25 to 45 years. Among the pheromones, ER-830 produced the most significant effect ($\bar{X} = 18.2$ mV·s; SEM = 0.8; $P < 0.01$) with no significant differences within the group. The mean response amplitudes to clove oil and to diluent alone are similar in male and female subjects, and none of the male subjects reported detecting odor of the test substances.

Effects of human pheromones on the EOG

The summated receptor potential from stimulation of the OE was recorded in 9 human subjects: 3 males and 6 females. As described above, all substances were tested at the same concentration (25 pg) and were delivered in 1 s air pulses. However, in the experiments they were intercalated with a continuous flow of air (0.3 ml/s). The inset in Fig. 5 shows a typical olfactory potential recorded from a 37-year-old man following stimulation with clove oil. Stable recordings were obtained from the olfactory mucosa in all subjects utilizing this traditional olfactant. The chart in

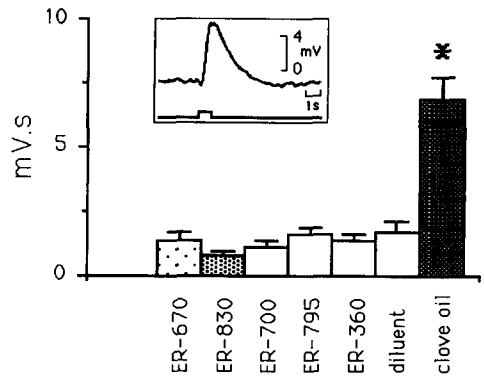

Fig. 5. Effect of pheromones and control substances on the EOG of a 37-year-old male. 25 pg of clove oil produces significant stimulation of the receptors (inset) ($P < 0.001$), while pheromones and diluent administered in the same quantity have a lesser effect.

Fig. 5 shows the mean effect produced in 9 subjects by the 5 putative pheromones, the diluent alone, and by clove oil. Stimulation with clove oil (25 pg) produced a large response ($\bar{X} = 6.8$; SEM = 0.9; $P < 0.001$) similar to those previously described [34, 41, 43, 44]. This was accompanied by a clear olfactory sensation in all subjects. While the diluent depolarized the olfactory receptors to a lesser extent than clove oil ($\bar{X} = 1.7$; SEM = 0.96), it also produced an olfactory sensation in all subjects. In contrast the putative pheromones have a minimal effect on the olfactory epithelium. The mean of the effects ranged from 0.79 to 1.58 mV·s. All subjects were questioned about odorant sensations following each stimulus, 7 did not report any olfactory sensation and 2 subjects, 1 male and 1 female, associated ER-795 and ER-700 to unpleasant odors. This finding reveals that at the concentrations used in our work, pheromones are not effective stimulants of olfactory receptors, but are stimulatory to the VN receptors.

Other effects of human pheromones

Studies have been initiated to determine how depolarization of the VN chemoreceptors by pheromones activates structures in the central nervous system (CNS) that could trigger reflex activity. We have attempted to correlate the EVG, parietal EEG events (Cz-A1 of the 10/20 system), and electrodermal activity (GSR) of the hand. The results obtained from a 36-year-old male subject are shown in Fig. 6. A single 500 ms air pulse of ER-830 with a concentration of 25 pg delivered to the VNO produced a large depolarization (6 mV) with a latency of 200 ms.

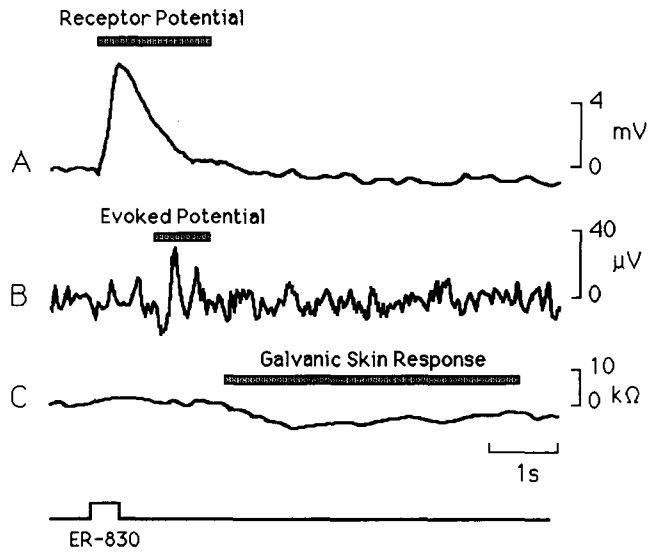

Fig. 6. Simultaneous recordings of EVG, cortical evoked potentials and GSR in a 36-year-old man. ER-830 (25 pg) applied to the VNO stimulated the receptors while synchronizing the EEG and decreasing skin conductance.

This was followed by an EEG evoked potential initiated by a positive deflection having a latency of 600 ms. This was followed by two or more negative events with a duration of 500 ms. There was also a change in skin conductance which appeared with a delay of 3 s. Similar results were obtained in 6 other subjects. These preliminary findings strengthen the concept that pheromonal activation of the human VNO may trigger reflex activity at multiple sites and may involve routes which are different from those employed by most olfactory stimuli.

DISCUSSION

EVG

Remarkably little information is available concerning the electrophysiological properties of receptors in the VNO. An early study of the mammalian VN system was initiated by Adrian [45] in 1954, who reported synchronous discharges from the rabbit VN nerve to mechanical stimulation. This was followed by an investigation of electrical activity of the VNO in reptiles [7, 8]. Our studies of the rat VNO nerve *in vitro* preparation [40] were started in 1990 and involved direct stimulation of sensory epithelium of the VNO with minute quantities of chemicals. Local negative potentials were observed, presumably to represent the summated receptor potential of chemoreceptors which were correlated with discharges of the VN nerve. This system provided for the application of known concentrations of a variety of olfactory stimuli and putative pheromones to a mammal with a well-developed VN system where the importance of the VNO for reproductive behavior is well-known.

The present investigation attempted to characterize for the first time chemically induced electrical potentials in humans by means of a non-invasive method. Our results show that a local response originating in the sensory elements of the VN epithelium can be elicited with the same stimuli which were effective in activating the rat VN organ. Such activity is independent of a contribution of olfactory receptors to chemically induced receptor potentials. Thus, the present investigation confirms that similar receptor potentials can be generated by chemosensory stimuli of the VNO in both the human and the rat. Further, in both the human and the rat there are characteristic and independent responses of the VNO and the OE. There is also preliminary evidence that the EVG does not arise from trigeminal nociceptor endings since application of a local anesthetic (lidocaine) to the respiratory epithelium of the nasal septum neither blocks nor diminishes the EVG in human subjects [28, 41, 43, 46, 47]. Furthermore, the EVG lacks the positive component which has been associated to trigeminal electrical potentials involving nasal nociceptors in neurophysiological studies of the olfactory mucosa [28, 44].

Our findings show that the receptor potential amplitude is strictly dependent both on stimulus intensity and stimulus duration which is in

agreement with Ottoson's [32, 34] classical study of the properties of vertebrate olfactory receptors. Thus, the slow decrement of VNO receptor potentials in response to continuous stimulation indicates that the receptors are of the slowly adapting type and the fact that repetitive stimulation of the VNO led to a decremental response while serial stimulation with different test substances elicits larger potentials also accounts well with known properties of olfactory receptors. The former phenomenon, known as selective fatigue, could play an important role in pheromonal discrimination at the level of the receptor organ.

In summary, it is clear that the general properties of the human EVG are similar to the potential recorded from the rat VN organ [40] and that both correspond to the summated receptor potential of neuroepithelial cells lining the lumen of the organ [30, 32, 34, 40, 46].

Effect of putative pheromones

The fact that stimulation of the VNO with different substrates resulted in different potential patterns suggests the existence of specific receptor sites with different sensitivities. For example, using equimolecular stimuli, VNO receptors are clearly more sensitive to putative pheromones than to olfactants while the opposite is true for olfactory receptors. Thus, while both structures may have receptor sites which recognize putative human pheromones, the response pattern is clearly different for these two sensory tissues.

In humans, sexually dimorphic responses to olfactants and hormone metabolites have been reported, although the results are contradictory [42, 48]. However, the methods of stimulation used in these experiments [42, 48] do not exclude the possibility that such stimuli could have not only activated olfactory receptors but may have also stimulated the VNO. In our experiments, sexual differences were noted in the effects of two putative pheromones ER-670 and ER-830, which appear to indicate receptor sexual dimorphism. In view of the fact that the VN system in animals shows marked sexual differences [3, 9], the finding of gender-specific effects among our test substances is not surprising. However, the fact that such differences are more prominent in the VNO of the human species than in the rat was a novel and unexpected finding. In animals the VNO has specific connections to the medial preoptic area of the hypothalamus and to parts of the limbic system [3, 9, 20, 22, 35]. These paths are part of the neuroendocrine LHRH system that develops in early stages of gestation and is involved in sexual chemical dimorphism [10, 24, 49–52]. Clinical evidence indicates that a similar role could exist in humans [53–55]. On the other hand, preliminary findings suggest that it is possible to activate components of the autonomic nervous system in humans. Changes in skin conductance have been observed in male as well as female subjects, which indicates that the same system could be active in humans [41]. Furthermore, our experiments suggest that stimulation of peripheral receptors located in the VNO with specific substances (Fig. 6) produce synchronization of the EEG in an area known to have olfactory afferents [44, 56]. There is also activation of the autonomic nervous system revealed by a change in skin conductance. Thus, while the precise nature of the relation of the human VNO with the CNS remains to be elucidated, the evidence indicates that the system responds to a variety of chemosensory stimuli and that some are able to induce reflex autonomic activity.

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