Human odor intensity perception: correlation with frog epithelial adenylate cyclase activity and transepithelial voltage response

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(Accepted 13 March 1990)

Key words: Adenylate cyclase; Cyclic adenosine 3',5'-monophosphate; Electro-olfactogram; Odor perception; Olfactory epithelium; Psychophysics; Multidimensional scaling

Although a number of odorants are hypothesized to depolarize frog olfactory receptor cells by binding to ciliary glycoproteins which activate membrane-bound G-proteins to induce adenylate cyclase-mediated increases in intracellular cAMP (cyclic adenosine 3',5'-monophosphate), it is not known whether these odorants influence human odor perception via similar mechanisms. In this paper we present evidence derived from odor attribute ranking and multidimensional scaling procedures that the perceived intensity of such odorants to humans is correlated with (a) the amount of adenylate cyclase activity they induce in an in vitro frog olfactory cilia preparation and (b) the magnitude of their influence on the frog transepithelial voltage response or electro-olfactogram (EOG). These observations are in accord with the hypothesis that the perception of the intensity of some odors by humans is associated with cAMP-related epithelial processes and imply that remarkable homologies exist between the intensity-related olfactory receptor mechanisms of frog and man.

INTRODUCTION

The physiological processes by which humans detect, interpret, and respond to odorants are poorly understood, as evidenced by the dozens of diverse theories proposed to explain human olfactory perception. No unitary relationship analogous to that found in vision between photic wavelength and color or in audition between vibration frequency and pitch has been demonstrated for the sense of smell, and large individual differences have been noted in smell ability. Furthermore, numerous observations point to this sense's inherent complexity. For example, olfactory receptor cells are individually responsive to many types of odorants and serve not only as the receptor elements, but as the first-order neurons of the primary olfactory pathway. Of particular interest to neurobiologists is the fact that such nerve cells are in a state of flux, undergoing periodic death and replacement.

Recent biochemical studies of frog and rat olfactory epithelia suggest that vertebrate odorant receptors are likely to be glycoproteins localized on the sensory cilia that extend into the mucus from the dendritic knob of the primary olfactory neurons. Odorants which bind to such receptors are hypothesized to activate a membrane-bound G-protein which, in turn, induces an adenylate cyclase-mediated increase in intracellular cyclic adenosine 3',5'-monophosphate (cAMP). Increased levels of cAMP then lead directly to an increase in ion conductance through gated membrane channels, thereby depolarizing the cells.

The question whether adenylate cyclase-mediated epithelial processes correlate with any element of human olfactory experience has yet to be answered. If (a) the level of adenylate cyclase activity induced by odorants in an in vitro preparation of frog olfactory cilia reflects the relative number of cells activated and/or their firing rates and (b) analogous adenylate-cyclase mediated processes are present in humans, then a positive correlation would be expected between the level of adenylate cyclase activity induced by an odorant in such a preparation and its perceived intensity by humans. Alternatively, if the general level of odor-induced adenylate cyclase activity in a ciliary preparation is associated with some other dimension of human odor perception (e.g., a non-intensity dimension derived from multidimensional scaling (MDS) analysis of odor similarity ratings), then relationships between adenylate cyclase activity and such

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results

Relatively robust relationships were present between the average intensity rankings of the subjects and both the odor-induced adenylate cyclase activity and the EOG value rankings (respective group data Spearman r's = 0.58 and 0.76, Ps < 0.001)37. Furthermore, such associations were consistent across subjects. Thus, all 20 Spearman r's computed between the intensity rankings of each subject and the adenylate cyclase activity value rankings were positive, as were all 20 Spearman r's computed between the intensity rankings and the EOG value rankings (binomial test P's < 0.0001). Despite such consistency, however, considerable variation was present among individuals in the magnitude of the association between the intensity data and the enzyme and EOG measures. This variation was present even though the judgment task was reliably performed by all subjects (Table I). An examination of the relationships between (a) the human intensity rankings and (b) the enzyme and EOG data revealed that, in 16 of the 20 subjects, a stronger relationship was present with the EOG data than with the adenylate cyclase activity data (P < 0.01, binomial test).

To explore whether specific anosmias might be present...
TABLE II

Correlations following removal of outliers

Spearman r values following removal of data from subjects who had atypically low rankings for some odorants that may have been indicative of specific anosmias. Values for rintensity,enzyme between ranked odor intensities and odor-induced adenylate cyclase activity values published by Sklar et al.38. Values for rintensity,EOG between ranked odor intensities and frog EOG magnitude values published by Lowe et al.21.

<table>
<thead>
<tr>
<th>Subject code</th>
<th>rintensity,enzyme</th>
<th>rintensity,EOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KB</td>
<td>0.78**</td>
<td>0.68**</td>
</tr>
<tr>
<td>IF</td>
<td>0.50*</td>
<td>0.55*</td>
</tr>
<tr>
<td>KL</td>
<td>0.27</td>
<td>0.20</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MH</td>
<td>0.73**</td>
<td>0.82***</td>
</tr>
<tr>
<td>JC</td>
<td>0.57*</td>
<td>0.86***</td>
</tr>
<tr>
<td>LL</td>
<td>0.66*</td>
<td>0.55*</td>
</tr>
<tr>
<td>MR</td>
<td>0.49*</td>
<td>0.64*</td>
</tr>
<tr>
<td>SN</td>
<td>0.43</td>
<td>0.75*</td>
</tr>
<tr>
<td>Overall</td>
<td>0.60*</td>
<td>0.77*</td>
</tr>
</tbody>
</table>

***P < 0.001: **P < 0.01; *P < 0.05

in a few subjects which would attenuate some of the correlations presented in Table I, we computed, separately for each odorant, the mean and standard deviation of the rank values (which, on the basis of the central limit theorem, would be expected to be normally distributed). This was done separately for each sex and responses which fell 1.64 S.D. units below the mean were identified. We then removed the outliers from the data set and recalculated the correlation coefficients. In the 8 subjects whose rankings contained the outliers, there was no consistent change in either the magnitude or the direction of the rintensity,enzyme and the rintensity,EOG (Table II), implying that the presence of extreme values was not the basis for the less robust correlations (Table I).

A three-dimensional MDS solution was found to best represent the data for the 8 subjects in the second phase of the study, with the average r² and stress (Young’s S-stress formula 1) values for the 8 matrices being 0.75 and 0.19, respectively. The first dimension obtained in this analysis correlated well with the enzyme activity (r = −0.77), the EOG magnitude (r = −0.59), and the average intensity rankings of the 8 subjects (r = −0.72); the pleasantness rankings exhibited only a weak correlation with this dimension (r = −0.21). The second dimension of this analysis did not meaningfully correlate with either the enzyme, EOG, or intensity measures (respective r’s = 0.17, 0.01 and 0.03), but did correlate strongly with the pleasantness rankings (r = 0.89). The third dimension evidenced no strong relationships with any of these measures (intensity, pleasantness, enzyme, and EOG r’s = −0.45, −0.22, 0.17, and −0.29, respectively).

DISCUSSION

The present findings lend support to the hypothesis that cAMP-related processes associated with olfactory transduction are present in both frog and man and imply that the perception of the intensity of a number of odorants may be a direct reflection of the amount of cAMP activity occurring at the receptor level. Furthermore, these findings are in accord with the notion, suggested by Otoson in 1971 on the basis of EOG responses, that the physiological activity within the frog olfactory epithelium is remarkably similar to that within the human olfactory epithelium. However, even though the mechanisms by which odor intensity is coded for the selected odorants appear to be analogous in these two forms, it is still possible that these species have fundamentally different sets or distributions of sets of olfactory receptor proteins (which presumably code odor quality), resulting in disparate odor experiences. Unfortunately, no appropriate behavioral data related to the frog’s olfactory ‘Umwelt’ are available to shed light on this issue.

It should be emphasized that the results of the present study are correlational and, thus, cannot be viewed as a definitive test of a causal association between adenylate cyclase activity and the perception of intensity. In addition to their relationship to adenylate cyclase activity, the intensity rankings were also correlated with a number of physicochemical parameters, including the octanol:water partition coefficient (r = 0.45) and molecular weight (r = 0.51). Although the first of these correlations implies an association between perceived intensity and hydrophobicity, it is not clear whether, in fact, this is a causal association, since a number of physicochemical parameters are strongly interrelated and this could therefore reflect an epiphenomenon. Furthermore, a myriad of physicochemical parameters are presumably associated with an odorant’s perceived intensity and ability to bind to receptor sites. However, within the theoretical framework of the hypothetical link between G-proteins and adenylate cyclase activity, the association between adenylate cyclase activity and such variables as perceived intensity and EOG magnitude is clearly in accord with the hypothesis that some aspects of human olfactory function are mediated by adenylate cyclase-related processes.

The basis for the individual differences in the magnitude of the correlations between the intensity values and both the enzyme and EOG data is not known. While it is tempting to hypothesize that such differences are related to the large variations in olfactory sensitivity observed among individuals in the general population, a more thorough understanding of the number and types of
biochemical transduction systems involved in the initial olfactory membrane events is needed before such an hypothesis can receive adequate test. The fact that a stronger relationship was present between the intensity rankings and the EOG data than between the intensity rankings and the adenylate cyclase activity data suggests the possibility that transduction mechanisms in addition to those associated with cAMP formation are associated with intensity-related processes. If cAMP is only one of several possible systems (some of which may not involve second messengers or even receptors, per se), the individual differences could arise from differential involvement of one or some combination of these systems.

Although it is also conceivable that psychophysical procedures more sophisticated than that of a simple ranking of perceived intensity might lead to less individual variation and to the elucidation of stronger associations between the variables, we explored the use of magnitude estimation and cross-modal matching procedures in pilot work and found relations similar to those obtained using the ranking procedure.

Although the present data are congruent with findings that Gs-deficient pseudohypoparathyroid (PHP) patients evidence impaired olfactory function (in support of the idea that cAMP associated G-proteins are involved in some aspect of human odor perception), recent research suggests that a G-protein other than Gs may be involved in olfactory signal transduction, per se. Thus, additional research is needed to clarify the role of Gs in olfactory perception and to establish whether the olfactory dysfunction of PHP patients is, in fact, directly associated with Gs-related adenylate cyclase associated mechanisms within the olfactory neuroepithelium proper.

**Acknowledgements.** Supported by National Institute on Deafness and Other Communication Disorders Grant POI 00161 and Grant AG 08148 from the National Institute on Aging. We thank Gary Burlingame, Geoffrey Gold, David Marshall, David Moran, Robert Mair, John Pierce, R. Gregg Settle, and Mel Suffet for their suggestions and assistance, and Jonathan Pevsner for kindly providing a number of the test stimuli.

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