Olfactory Dysfunction in Type I Pseudohypparathyroidism: Dissociation from G\textsubscript{s}α Protein Deficiency*

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ABSTRACT

The discovery of variably decreased olfactory ability in Type Ia pseudohypparathyroidism (PHP), a syndrome in which generalized hormone resistance is associated with deficiency of the alpha chain of the stimulatory guanine nucleotide-binding protein (G\textsubscript{sa}) of adenylyl cyclase, has been used to support the hypothesis that G\textsubscript{sa} plays a major role in human olfactory transduction. However, only a limited number of olfactory tests have been administered to such patients, and these patients have other problems that might cause or contribute to their olfactory dysfunction, including an unusual constellation of skeletal and developmental deficits termed Albright hereditary osteodystrophy (AHO). In this study, we administered tests of odor detection, identification, and memory to (i) 13 patients with Type Ia PHP; (ii) 8 patients with Type Ib PHP; (iii) 7 patients with pseudohypparathyroidism (PPHP); and (iv) 3 sets of normal controls matched to these groups on the basis of age, gender, and smoking history. Although we confirm that PHP Type Ia patients evidence olfactory dysfunction, we also demonstrate that (i) patients with Type Ib PHP, who have no AHO, no generalized hormone resistance, and normal G\textsubscript{sa} activity, also evidence olfactory dysfunction relative to matched controls; and (ii) patients with PPHP, who have AHO, no generalized hormone resistance, and deficient G\textsubscript{sa} protein activity, have relatively normal olfactory function. These observations do not support the hypothesis that the olfactory dysfunction associated with PHP is the result of generalized G\textsubscript{sa} protein deficiency and imply that other mechanisms (e.g., ones associated with PTH or PTHrP resistance) are responsible for the olfactory deficits of this disorder. (J Clin Endocrinol Metab 82: 247–250, 1997)

Numerous hormones, neurotransmitters, and cytokines regulate cell function by stimulating production of the intracellular signalling molecule cAMP (1). These ligands bind to specific cell surface receptors that are coupled to membrane-bound adenylyl cyclase molecules by heterotrimeric G proteins. Agonist-bound receptors activate G proteins by promoting exchange of GTP for GDP bound to the α subunit of the heterotrimer. The α-GTP molecule rapidly dissociates from the βγ complex and can interact with its effector until its intrinsic GTPase hydrolyzes it to GDP, thus promoting reassociation of α-GDP with βγ. In most cells, receptors are coupled to activation of one or more forms of adenylyl cyclase by G\textsubscript{sa}, (2).

It has been suggested that G\textsubscript{sa} may play an important role in olfactory transduction, although a genetically distinct G protein, termed G\textsubscript{olp}, likely couples olfactory receptors to a unique form of adenylyl cyclase (3). Support for a role of G\textsubscript{sa} in olfactory function comes from results that G\textsubscript{sa}-deficient Type Ia pseudohypparathyroidism (PHP) patients, who express generalized hormone resistance, evidence variably decreased olfactory ability relative to non-G\textsubscript{sa} deficient Type Ib PHP patients, whose hormone resistance is specific to parathyroid hormone (PTH) (7, 8). Although strong positive correlations between intensity ratings given to odors by humans and the propensity of such odors to stimulate adenylyl cyclase activity in an in vitro frog olfactory ciliary preparation add further credence to the role for G-proteins in human olfactory transduction (9), such findings do not shed light on the specific G proteins involved.

While the decreased olfactory function in PHP Type Ia patients seems to provide compelling evidence for a role of G\textsubscript{sa} in human olfactory processing, few types of olfactory tests have been administered to these unusual patients, and they have other problems that might explain or contribute to their decreased ability to smell. For example, Type Ia PHP patients, unlike Type Ib PHP patients, have Albright hereditary osteodystrophy (AHO), an unusual constellation of skeletal and developmental abnormalities that includes obesity, short stature, brachydactyly, round faces, and subcutaneous ossifications (10).

To better define the apparent association between G\textsubscript{sa} protein deficiency and olfactory dysfunction, we administered odor identification, detection, and memory tests to Type Ia and Type Ib PHP patients, as well as to patients with pseudohypparathyroidism (PPHP) and to normal subjects matched to the patients on the basis of age, gender,
and smoking habits. PPHP is a condition sometimes found in relatives of patients with PHP Type Ia and shares with Type Ia PHP the presence of AHO and decreased G\(_\alpha\) protein activity. However, PPHP is unaccompanied by generalized end organ insensitivity to hormones. Our results confirm that PHP Type Ia patients score lower on an odor identification test than do patients with PHP Type Ib. However, we also demonstrate (i) that PHP Type Ib patients, relative to matched controls, evidence deficits in olfactory function and (ii) that G\(_\alpha\) protein deficient PPHP patients have relatively normal olfactory function. These observations do not support the hypothesis that the olfactory dysfunction associated with PHP is the result of a generalized deficiency of G\(_\alpha\) protein.

**Materials and Methods**

**Subjects**

Thirteen patients with Type Ia PHP, 8 patients with Type Ib PHP, and 7 patients with PPHP served as subjects, along with 3 groups of normal controls whose members were matched individually to each of the patients on the basis of age, gender, and smoking history (Table 1). One Type Ia PHP patient did not receive the odor detection threshold test, and another did not receive the odor memory test (see Olfactory Test Procedure section). Most of the patients were recruited from families with multiple affected members who were followed by M.A.L. and A.M. at Johns Hopkins University and the State University of New York, Syracuse, respectively. Two were patients of Robert Rosenfield, M.D., of Wyler Children’s Hospital in Chicago, and one of Andrew Stewart, M.D., of the Veteran’s Affairs Medical Center, West Haven, Connecticut. Four were patients of Luis Aparicio, M.D., of Metabolic Associates, Erie, Pennsylvania. All of the patients provided informed consent to be tested and were tested in their homes by A.D.F. or D.A.M. The diagnoses of Type Ia PHP, Type Ib PHP, and PPHP were based on determination of hormone responsiveness, G\(_\alpha\) activity, and the presence or absence of AHO, as previously described (11, 12). Erythrocyte membrane G\(_\alpha\) was analyzed using either a bioassay to measure activity (12) or by a quantitative immunoblot technique to determine the relative level of immunoreactive protein (13), as described below. Results for many of the patients evaluated in this study were reported previously (12–15). Demographic information, as well as mean (sd) G\(_\alpha\) erythrocyte levels and scores on the Mini-Mental State Examination (16), a measure of cognitive function, and the Picture Identification Test (17), a visual test designed to control for nonolfactory elements of the odor identification test used in this study, are presented in Table 1.

**Measurement of membrane G\(_\alpha\) bioactivity**

Assays of membrane G\(_\alpha\) bioactivity were performed as previously described (12), based on activation of adenylyl cyclase in membranes from the ccy\(_c\) clone of the S49 murine lymphoma cell line, which genetically lacks G\(_\alpha\) (18). Membranes from S49 ccy\(_c\) cells were prepared as previously described (19).

**Quantitative immunoblot analysis**

Membrane proteins (50–100 \(\mu\)g protein) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using the system of Laemmli (20). In order to optimize resolution among G proteins, membrane samples were alkylated with N-ethylmaleimide (21) before electrophoresis through 11% acrylamide gels. The separated proteins were transferred from the gel to PVDF membranes (Millipore) using a Transblot chamber (Bio-Rad). We performed immunoblot analysis using specific polyclonal rabbit antisera that had been raised against synthetic peptides corresponding to defined regions of the G\(_\alpha\) protein (i.e. antiserum C564 was raised against a peptide [CTPEGEDPRYTRAKY] consisting of residues 325–339 of G\(_\alpha\), and antiserum RM was raised against the c-terminal decapeptide [RMHLRYELF] corresponding to residues 385–394). Both antisera recognize the 45 kDa and 52 kDa forms of G\(_\alpha\). Each immunoblot was incubated with a saturating concentration of the appropriate antiserum, and antibody binding was detected by subsequent incubation with \(^{125}\)I-labeled protein A and autoradiography. The optical density of each band was measured by two-dimensional scanning densitometry, and regions of the blot that corresponded to the bands were excised and the amount of radioactivity was quantitated by \(\gamma\)-spectrometry. Over the range of protein concentrations used in these studies, optical density was proportional to the amount of radioactivity associated with each antigen band. In all cases presented, there was a linear relationship between the amount of membrane protein loaded onto the gel and the amount of immunoreactive signal detected.

**Olfactory test procedures**

The patients and matched controls were administered standardized tests of odor identification, detection, and odor memory. The order of presentation of the tests was randomized across subjects. The test session lasted approximately an hour and a half, and the subjects were allowed to take rest breaks between the administration of the tests.

The first test that we administered was the University of Pennsylvania Smell Identification Test (UPSIT; commercially available as the Smell Identification Test, Sensonics, Inc., Haddon Hts., NJ). This 40-item microencapsulated test is the most widely-administered test of olfactory function in North America (having been administered to an estimated 30,000 patients) and is described in detail elsewhere (22, 23). Briefly, a subject is required to identify, in a 4-alternative multiple choice format, each of 40 odors presented on microencapsulated “scratch and sniff” labels. For example, one of the test items reads, “This odor smells most like: (a) chocolate; (b) banana; (c) onion; or (d) fruit punch.” The subject must provide a response even if no odor is perceived (i.e. the test is forced-choice). The number of items out of 40 that were answered correctly served as the dependent measure.

The second test we administered was the phenyl ethyl alcohol (PEA) odor detection threshold test. In this test, detection threshold values for PEA, a rose-like smelling odorant with minimal intranasal trigeminal nerve activity, were determined using a modified single staircase procedure described in detail elsewhere (22). In the present study, the staircase was begun at the −6.50 log concentration step of a half-log step (vol/vol) dilution series extending from −10.00 log concentration to −2.00 log concentration and was moved upward in full log steps until correct detection occurred on five sets of consecutive trials at a given

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**TABLE 1. Description and demographics of study population**

<table>
<thead>
<tr>
<th>Group</th>
<th>Males/Females (no.)</th>
<th>Age</th>
<th>Current, Previous, &amp; Never Smokers (no.)</th>
<th>Mini-Mental State Examination</th>
<th>Picture Identification Test</th>
<th>G(_\alpha) (% of normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHP-Ia</td>
<td>10/3</td>
<td>25.42 (14.85)</td>
<td>2, 1, 10</td>
<td>26.50 (3.40)</td>
<td>38.17 (2.04)</td>
<td>46.42 (8.86)</td>
</tr>
<tr>
<td>PHP-Ib</td>
<td>6/2</td>
<td>44.63 (14.94)</td>
<td>1, 0, 7</td>
<td>29.38 (1.06)</td>
<td>40.00 (0)</td>
<td>94.50 (9.40)</td>
</tr>
<tr>
<td>PPHP</td>
<td>0/7</td>
<td>40.29 (11.80)</td>
<td>4, 2, 1</td>
<td>29.00 (0.82)</td>
<td>40.00 (0)</td>
<td>53.67 (7.61)</td>
</tr>
<tr>
<td>II. Normal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHP-Ia</td>
<td>10/3</td>
<td>25.39 (13.17)</td>
<td>2, 1, 10</td>
<td>29.50 (0.84)</td>
<td>39.77 (0.60)</td>
<td>ND</td>
</tr>
<tr>
<td>PHP-Ib</td>
<td>6/2</td>
<td>45.50 (13.65)</td>
<td>1, 0, 7</td>
<td>29.67 (0.58)</td>
<td>40.00 (0)</td>
<td>ND</td>
</tr>
<tr>
<td>PPHP</td>
<td>0/7</td>
<td>40.71 (12.51)</td>
<td>4, 2, 1</td>
<td>28.75 (2.50)</td>
<td>39.86 (0.38)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Numbers in parentheses reflect standard deviations. G\(_\alpha\) is erythrocyte G\(_\alpha\) protein activity. See text for details. ND, no data.
concentration. If an incorrect response was given on any trial, the staircase was moved upward a full log step. When a correct response was made on all five trials, the staircase was reversed and subsequently moved up or down in 0.50 log increments or decrements, depending upon the subject’s performance on two pairs of trials (each pair consisting of a choice between a blank and an odorant) at each concentration step. The geometric mean of the four staircase reversal points following the third staircase reversal was used as the threshold measure.

The third test of the test battery was one of odor recognition memory. On a given trial of this 12-trial test (see ref. 24 for details), a microencapsulated “target odorant” is presented to a subject, followed by four odorants from which the subject is instructed to select the one identical to the target stimulus. On four trials, a 10-second interval is interspersed between the sampling of the target stimulus and the presentation of the first of the four alternatives. On four others, a 30-second interval is enforced, whereas on the other four trials a 60-second period intervenes. In the present study the number of trials in which the target odor was correctly identified, irrespective of delay interval, served as the dependent measure.

**Results**

The mean (SEM) test scores for the three olfactory tests for the PHP Type Ia, PHP Type Ib, PPHP, and matched control subjects are presented in Figs. 1–3. Matched-pairs t-tests revealed that the Type Ia PHP subjects performed significantly more poorly than their matched controls on all three tests [t-tests with Bonferroni-corrected α levels: UPSIT t (12) = 11.08, P < 0.0001; PEA t (11) = 3.46, P < 0.05; memory t (11) = 6.20, P < 0.0001]. The Type Ib PHP patients were also less sensitive than the controls on the PEA odor detection threshold test [PEA t (7) = 6.64, P = 0.005] and on the odor memory test [t (7) = 3.48, P < 0.05], but not on the UPSIT [t (7) = 1.49, P > 0.20]. None of the scores of the PPHP patients differed significantly from those of their matched controls (all Ps > 0.50).

It should be noted that, while Type Ib PHP patients typically outperformed the Type Ia PHP patients on the olfactory tests, their matched controls did likewise. This suggests the possibility that the seemingly better performance of the Type Ib patients could be the result of nondisease-related variables. To establish whether the relative olfactory function of the Type Ib PHP patients was, in fact, superior to that of the Type Ia PHP patients, we performed t-tests across the patient test scores after they had been subtracted from those of their matched controls. These analyses revealed no significant differences between the Type Ia and Ib PHP patients for the PEA odor detection and odor memory tests [PEA t (18) = 0.99; memory t (18) = 1.17; all Ps > 0.20]. However, a significant difference did emerge for the UPSIT [t (19) = 6.29, P < 0.0001], implying that odor identification is more markedly altered in Type Ia patients than in Type Ib patients.

**Discussion**

The present study confirms earlier observations that Type Ia PHP patients evidence olfactory dysfunction (7, 8). However, this study also demonstrates (i) that patients with Type Ib PHP have some olfactory dysfunction relative to matched controls, and (ii) that Gα protein deficient PPHP patients have relatively normal olfactory function. Taken together,
these observations throw into question the hypothesis that the olfactory dysfunction associated with PHP Type Ia is caused by generalized $G_{olf}$ protein deficiency. Furthermore, these data clearly demonstrate that the loss is not the result of AHO, per se.

Although the basis for the olfactory deficits in Type Ia and Type Ib PHP patients is unknown, several possibilities are worthy of consideration. First, it is conceivable that these types of PHP patients have, perhaps to differing degrees, decreased function in the primary olfactory G protein, $G_{olf}$ or in the type III olfactory adenylyl cyclase that is coupled by $G_{olf}$ to odorant receptors (5, 6). Second, in as much as the olfactory adenylyl cyclase behaves as a coincidence detector that can be stimulated by both $G_{olf}$ and $Ca^{2+}$ (5), it is possible that mild hypocalcemia, which is common in patients with Type Ia and Type Ib PHP, may reduce generation of cAMP in olfactory neuroepithelium and thereby impair olfaction. Third, given that PHP Type Ia patients (i) seem to have greater odor identification dysfunction than PHP Type Ib patients and (ii) exhibit a wider range of hormone resistance than do PHP Type Ib patients, the difference in odor identification ability may be related to the degree of target tissue resistance to hormones that act via stimulation of the cAMP second messenger system. The failure to find this degree of difference on olfactory tests other than odor identification may reflect their relatively lower reliability and sensitivity, rather than the sampling of different neural substrates (25).

Fourth, given the normal olfactory function of the PPHP patients, who do not exhibit PTH resistance, the decreased olfactory function in the Type Ia and Ib PHP patients could reflect PTH resistance, per se. However, since no olfactory disturbance has been reported in other patients with PTH deficiency, such as patients with hypoparathyroidism, it is possible that PTRP resistance, rather than PTH resistance, is the mechanism responsible for the decreased olfactory function.

Whatever the basis for the olfactory alterations observed in Type I PHP, the present study clearly demonstrates that such losses are observed on several types of olfactory tests. While the influence of Type I PHP on nominally disparate olfactory tests could reflect the involvement of cAMP-related biochemical changes at multiple levels within the nervous system, alterations within limited sectors of the olfactory pathway could also explain this result. For example, if detection ability is altered as a result of biochemical changes within the olfactory epithelium, poor performance would also occur on tests of odor memory and identification, because no odor sensation would be available for encoding the percept needed for such tasks (26). Thus, in such cases, underlying neural circuits needed for odor memory or identification could still be intact. Clearly, research is needed to determine if the PHP-related changes in olfactory function reflect, in fact, alterations in the olfactory receptor cells themselves, or if alterations in other elements of the olfactory pathway are also present.

Acknowledgments

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References


