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SHORT COMMUNICATION

G-protein βγ Subunit Genes Expressed in Olfactory Receptor Neurons

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Abstract

The expression of genes encoding G-protein βγ subunits was investigated in isolated olfactory receptor neurons from channel catfish. DNA sequencing of PCR products showed that the β1, β2, γ2 and γ3 genes were expressed in the neurons. Western blotting showed that at least three of these subunit proteins were expressed. This first analysis of the expression of βγ genes in olfactory receptor neurons suggests that these subunits may be involved in a variety of transduction events in these cells. **Chem. Senses 22: 587–592, 1997.**

Introduction

Olfactory receptor cells are specialized neurons within the olfactory epithelium of vertebrates that detect odorants and transduce stimulus-encoded information into cellular responses. The receptor cells have several cilia protruding from their apical dendritic surfaces that are exposed to the external environment and serve as the contact sites of odorants with the receptor cells. Olfactory transduction is initiated at the cilia by the binding of odorants to members of a large multigene family of G-protein-coupled odorant receptors (Buck and Axel, 1991; Ngai *et al.*, 1993). Odorant binding to the receptors activates G-protein-linked second messenger systems that increase intracellular levels of cyclic AMP (Lancet, 1986) or phospholipid-derived second messengers (Bruch, 1996). These second messengers gate nonselective cation channels, thereby providing a mechanism for membrane depolarization, action potential generation and synaptic transmission to the central nervous system.

Heterotrimeric G-proteins are composed of three subunits of unequal molecular mass, designated α, β and γ. Biochemical purification and molecular cloning experiments have shown that all three subunits are the products of multigene families (Gilman, 1987; Simonds, 1994). In vertebrates, the α subunit gene family includes over 20 genes and splice variants, while the β and γ subunit families include five and 11 members respectively (Gudermann *et al.*, 1996). The expression of individual subunits may be ubiquitous or may be restricted in a tissue- or cell-specific manner (Simonds, 1994). For example, a novel γ subunit was recently described that is expressed in immature, but not

mature, receptor cells during neurogenesis in both the main olfactory epithelium and in the vomeronasal gland (Ryba and Tirindelli, 1995). Because of the central role of G-proteins in mediating olfactory transduction (Lancet, 1986; Bruch, 1996), the expression of members of the α subunit family has been previously investigated. At least six of these proteins are expressed in rat olfactory epithelium (Jones and Reed, 1987, 1989). In channel catfish, Western blotting and immunocytochemical studies showed that at least five α subunits are expressed in olfactory cilia (Abogadie *et al.*, 1995). Multiple α subunits have also been identified in frog olfactory epithelium (Pace and Lancet, 1986; Anholt *et al.*, 1987). Olfactory cilia also exhibit intense immunoreactivity when probed with an antibody that recognizes a conserved sequence shared by all members of the β subunit gene family (Anholt *et al.*, 1987; Abogadie *et al.*, 1995). However, the identification of specific β subunits, and their companion γ subunits, in olfactory receptor cells has not been described previously. These observations, together with the role of these subunits in activation of G-protein-coupled receptor kinases in isolated olfactory cilia (Boekhoff *et al.*, 1994) and their potential role in modulating the activity of additional transduction components in the receptor cells (Iniguez-Lluhi *et al.*, 1993; Exton, 1996), prompted us to investigate the expression of genes encoding these subunits in olfactory neurons. Using degenerate primers complementary to conserved sequences of the β and γ subunit families in the polymerase chain reaction (PCR), we identified two members of each gene family. Western blots of isolated olfactory membrane preparations probed with subunit-specific antibodies showed that at least three of the subunits identified by PCR were expressed at the protein level in olfactory rosettes. To our knowledge, these results are the first characterization of the expression of these genes in olfactory receptor cells.

Methods, results and discussion

Isolated olfactory neurons were obtained as previously described (Bruch and Medler, 1996) using the papain dissociation procedure described by Restrepo and Teeter (1990). Dissociated cells were identified morphologically and harvested with a siliconized microcapillary. Cell lysis, isolation of poly(A)$^+$ RNA and reverse transcription were performed as previously described (Bruch and Medler, 1996). PCR amplification of β subunit sequences was performed with cDNA from three olfactory neurons using the degenerate primers E1 and E5 (Von Weizsacker, 1992). Amplification of γ subunit sequences was also performed with cDNA from three neurons using the degenerate primers G1 and G2 (Gallagher and Gautam, 1994). All PCR reactions were initiated by the addition of Taq polymerase at 90°C and were terminated after 35 cycles with a final extension at 72°C for 10 min followed by incubation of the samples at 95°C for 15 min. The samples were then cooled slowly over several minutes to 4°C. PCR products were cloned by TA cloning, and plasmids were screened and purified as previously described (Bruch and Medler, 1996). DNA sequencing was performed on both strands with the Fidelity sequencing system (Oncor, Gaithersburg, MD). G-protein subunit sequences were identified by comparison to sequences in the data banks using the BLAST program (National Center for Biotechnology Information, Bethesda, MD). Predicted amino acid sequences were obtained using the Gene Runner program (Hastings Software, Hastings, NY).

Our initial strategy to identify genes encoding G-protein βγ subunits in olfactory receptor neurons was to use degenerate primers in the PCR to amplify conserved sequences shared by members of each gene family (Von Weizsacker *et al.*, 1992; Gallagher and Gautam, 1994). For each gene family, the reverse transcribed cDNA from isolated neurons was used for the PCR. The morphological characteristics of the isolated receptor cells have been described previously (Bruch and Medler, 1996). Analysis of the PCR reactions by agarose gel electrophoresis revealed a readily visible 240 bp band for the β subunits and a 140 bp band for the γ subunits (data not shown). Since the sizes of the PCR products corresponded to those expected (Von Weizsacker *et al.*, 1992; Gallagher and Gautam, 1994), the PCR products were cloned and sequenced to determine their identities. For the β subunits, 15 clones were obtained. At the nucleotide level, two clones were 70% identical to the bovine β1 subunit and 13 clones were 78% identical to the human β2 subunit. Predicted amino acid sequences were obtained from the nucleotide sequences and were used to compile consensus sequences for the β subunit clones. This analysis showed that the catfish β1 and β2 PCR products were 89 and 98% similar to their mammalian counterparts respectively (Figure 1). For the γ subunits, 14 clones were obtained. At the nucleotide level, three clones were 76% identical to the bovine γ2 sequence and 11 clones were 79% identical to the bovine γ3 sequence. Consensus amino acid

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β1 Fish    AAELKNQIRDARKASGDSTLTQITNALDPVCRIQMRT
   Bovine  E**************C**********N****G******

           RRTLRGHLGKIYAMHW
           ****************

β2 Fish    QEAEQLKNQIRDARKACGDSTLTQIRAGLDPVGRIQM
   Human   ***************************T***********

           RTRRTLRGHLGKIYAMHW
           ******************


γ2 Fish    VEQLKMEANIDRIKVSKAAADLMSYCDMHAKEDPLLS
   Bovine  ****************************A*********

           PVPATEN
           *******

γ3 Fish    VEQLKIEASFCRIKVSKAAADLMAYCDLHACEDPLIT
   Bovine  *********L*****************A*********

           PVPTSENPSRE
           ********F**
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Figure 1 Consensus amino acid sequences for G-protein βγ subunit PCR products. The amino acid sequences of the catfish PCR products are aligned with the corresponding regions of the mammalian sequences obtained from Genbank. Asterisks (*) indicate identical or conservatively substituted residues.

sequences for these clones were 98 and 92% similar to the bovine sequences for the γ2 and γ3 subunits respectively (Figure 1).

Western blotting was used to determine whether the proteins corresponding to the subunits identified by PCR were expressed, and if so, to determine whether any of the subunits were preferentially expressed in olfactory cilia. Isolated cilia and deciliated membrane preparations were prepared as previously described (Boyle *et al.*, 1987). Polypeptides in the cilia and deciliated membranes were resolved in 15% SDS–polyacrylamide gels with 4% stacking gels. The resolved polypeptides were transferred to Westran membranes (Schleicher & Schuell, Keene, NH) for immunoblotting. Western blotting was performed as previously described (Bruch and Abogadie, 1995). The blots were probed with 1:500 dilutions of affinity-purified, subunit-specific peptide antibodies to each of the four G-protein subunits identified by PCR (Santa Cruz Biotechnology, Santa Cruz, CA). The antibodies to the β1 and β2 subunits were produced to peptides corresponding to amino acids 25–40, a domain that is divergent in these subunits (Von Weizsacker *et al.*, 1992). The antibody to the γ2 subunit was produced to a peptide corresponding to amino acids 2–17, while the γ3 antibody was produced using a peptide corresponding to amino acids 2–21. The immunoblots showed that at least three of the proteins corresponding to the genes identified by PCR were expressed in olfactory tissue and were found in the isolated cilia as well as in deciliated epithelial membranes (Figure 2). Negative control blots that were not exposed to primary antibodies showed no immunoreactive bands. The expression of the β1 subunit protein could not be evaluated since blots probed with this antibody gave unacceptable background labeling.

Recent data indicate that G-protein βγ subunits are involved in signal transduction and modulate the activity of a variety of effectors, including adenylate cyclase, phospholipase C, ion channels and G-protein-coupled receptor kinases (Iniguez-Lluhi *et al.*, 1993; Simonds, 1994; Exton, 1996). Some of these effectors are regulated in a dual fashion by G-protein α subunits and by the βγ dimer. For example, phospholipase Cβ3 can be regulated by the α subunit of G_q and by the βγ dimer (Boyer *et al.*, 1994; Exton, 1996). Since the molecular nature of the odorant-sensitive

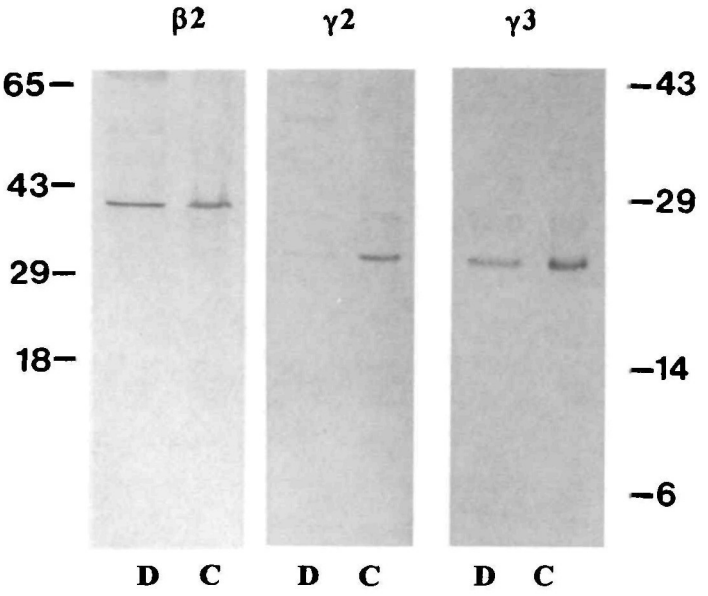

Figure 2 Western blots of G-protein βγ subunit proteins. Protein samples of deciliated epithelial membranes **(lanes D)** and isolated cilia **(lanes C)** were separated by SDS–polyacrylamide gel electrophoresis, transferred and probed with the indicated subunit-specific antibodies. Equal amounts (50 μg) of protein were loaded in each lane. For the β subunits, ~39 kDa immunoreactive bands were observed and for the γ subunits ~20 kDa immunoreactive bands were detected. In our gel system, the γ subunits apparently migrate as dimers, probably due to incomplete denaturation (Pronin and Gautam, 1994). For the β subunits the region of the gel from 65 to 15 kDa is shown and for the γ subunits the region of the gel from 45 to 5 kDa is shown.

phospholipase C has not yet been determined, it is possible that it may be linked to the odorant receptors by a G-protein α subunit and/or by the βγ dimer. In addition, Boekhoff *et al.* (1994) have shown in isolated olfactory cilia that translocation and activation of a G-protein-coupled receptor kinase is dependent on G-protein βγ subunits. These observations, together with previous immunocytochemical data indicating that olfactory cilia express abundant amounts of G-protein β subunits (Anholt *et al.*, 1987; Abogadie *et al.*, 1995), prompted us to investigate the molecular nature of the βγ subunits expressed in olfactory receptor neurons.

Of the 16 known genes encoding G-protein β and γ subunits, we identified two members of each gene family expressed in olfactory neurons by PCR. Since negative controls lacking reverse transcriptase showed no visible bands, it is likely that the PCR products were derived by reverse transcription of RNA, rather than nonspecific amplification of genomic DNA. In addition, the RNA used in these experiments was purified on oligo-dT to enrich the material in poly(A^+) RNA and hence minimized genomic DNA contamination. Because the PCR products of the β subunit, as well as of the γ subunit, cross-hybridize (unpublished observation), it was not possible to perform meaningful Northern blots or *in situ* hybridization to examine message distribution across the neuronal population. However, based on the high level of nucleotide and amino acid sequence similarity of the catfish PCR products with mammalian sequences, it can be concluded that the β1, β2, γ2 and γ3 subunit genes are expressed in catfish olfactory receptor neurons. Of the many possible combinatorial pairs of βγ subunits, not all pairs appear to be permissible. For example, in a recent study of the interaction of different β and γ subunits using the yeast two hybrid system, Yan *et al.* (1996) showed that γ1 failed to interact with β4 and β5. However, of the possible combinatorial pairs of the subunits we have identified in olfactory neurons, all four possible pairs were shown to interact in the yeast two hybrid system (Yan *et al.*, 1996). *In vitro* all four of these subunit pairs were shown to stimulate phospholipase Cβ from turkey erythrocytes, although the β2γ3 dimer was somewhat less effective than the other dimers (Boyer *et al.*, 1994). It is therefore possible that one or more of these dimers may be involved in regulating the activity of phospholipase C in olfactory neurons. Since all four possible subunit pairs are also capable of interacting with G-protein-coupled receptor kinases (Simonds, 1994), one or more of these dimers may also modulate the translocation and activation of these enzymes in olfactory neurons (Boekhoff *et al.*, 1994). However, we cannot rule out the possibility that additional βγ subunit genes are expressed in olfactory neurons or the possibility that the expression of these genes may differ across species.

Western blots were used to determine whether the corresponding proteins of the genes identified by PCR were actually expressed. Three of the subunits identified by PCR were detected at the protein level in isolated cilia and in deciliated epithelial membranes using subunit-specific antibodies to probe the blots. The finding of these polypeptides in the isolated cilia, which contains olfactory as well as nonchemosensory membranes, and the finding of these polypeptides in deciliated membranes suggest that these G-protein subunits probably mediate a variety of transduction events in response to extracellular signals in both the receptor cells and other cell types in olfactory rosettes. Ongoing experiments will specifically test the hypothesis that these subunits are functionally involved in olfactory signal transduction.

Previous studies have shown that the β1 and β2

subunit genes are ubiquitously expressed (Iniguez-Lluhi *et al.*, 1993; Exton, 1996). In contrast, the tissue distribution of some of the γ subunit genes appears to be more restricted. Previous studies indicated that the γ2 subunit was apparently expressed only in brain (Cali *et al.*, 1992; Simonds, 1994) and that the expression of the γ3 subunit was restricted to brain and retina (Simonds, 1994; Yan *et al.*, 1996). By demonstrating that the genes for both of these subunits are expressed in olfactory neurons, our results suggest that the expression of these subunits may be more general in neuronal systems than previously thought.

ACKNOWLEDGEMENTS

This work was supported by Louisiana Education Quality Support Fund grant RD-A-04 (to R.C.B.) and by NIH grant DC01500 (to R.C.B.). H.T and J.A.H. were supported by the Howard Hughes Medical Institute Undergraduate Research Program.

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Received on December 18, 1996; accepted on April 1, 1997