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Multiple genes for G protein-coupled receptors and their expression in lingual epithelia

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Using the polymerase chain reaction (PCR), we identified a gene family including more than 60 members which encoded similar G protein-coupled seven-transmembrane receptors. Sequence analyses of six representatives out of the 60 PCR clones showed that they had significant structural similarity to olfactory and optic receptors. Their expression is restricted in the surface of lingual epithelia.

Taste receptor; G protein-coupled receptor; Lingual epithelium; Polymerase chain reaction

1. INTRODUCTION

Taste transduction triggered by a variety of taste stimuli such as sweetness and bitterness is supposedly mediated by taste receptor cells in taste buds of the tongue [1–3]. Several lines of physiological evidence have revealed that taste signalling may be related to other types of sensory signalling such as phototransduction [4–6] and olfactory transduction [7,8] in terms of neurotransmitters [9,10] and second messengers including cyclic nucleotides (cAMP and cGMP) [11,12] and Ca^{2+} [13]. However, the molecular mechanism of taste transduction remains obscure, except for the recent identification of a novel taste-cell-specific G protein (α-gustducin) [14]. We have conducted a cloning study to identify some of the molecules possibly involved in taste transduction in rat. Here we describe the characterization of a gene family including more than 60 members which encode G protein-coupled receptors expressed on the apical surface of lingual epithelia.

2. MATERIALS AND METHODS

2.1. *Reverse transcription-polymerase chain reaction (RT-PCR)*

Poly(A^+)RNA was prepared from the tongue epithelia of Fischer rats using oligo(dT)-cellulose and ds-cDNA was synthesized according to the standard method [15]. The three oligonucleotide primers 5′-CC(GATC)ATGTA(TC)(TC)T(GATC)TT(TC)(TC)T(GATC)-TC(GATC)AA-3′, 5′- TG(GATC)GA(GATC)CC(AG)CA(GATC)-GT(GATC)GA(GA)AA-3′ and 5′-GA(AG)TA(AG)AT(AG)AA-(GATC)GG(AG)TT(GATC)A(AG)CAT-3′ were prepared according to the amino-acid sequences PMY(FL)FLSN, FSTC(AG)SH and MLNPFIYS commonly conserved in olfactory receptors [8] around transmembrane domains II, VI and VII, respectively. PCR was conducted with rat tongue epithelial cDNA with temperature cycles of 45 s at 96°C for denaturation, 2 min at 45°C for annealing, and 3 min at 72°C for extension, and the cycle number was 30. The amplified DNA fragments of ca. 500 bp were excised and cloned into the pUC18 vector. To obtain clones for G protein-coupled receptors from the mini-library, another oligonucleotide was prepared as a probe according to the sequence 5′- CTGTG(CT)(CC)AT(CT)GCIIT(GT)GA-(CT)(CA)G(CG)TAC-3′ that corresponded to a part of transmembrane domain III sequence LC(AV)IA(LVI)DRY [16].

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2.2. *Southern blot analysis*

Genomic Southern blotting was conducted using a 10-μg portion of rat liver DNA digested with *Eco*RI, *Hin*dIII or *Bam*HI. The blotted membranes were hybridized with ^{32}P-labelled probes for the six RT-PCR clones. The filter was washed at 68°C in 0.1 × SSC containing 0.1% SDS or washed at 50°C in 2 × SSC containing 0.1% SDS.

2.3. *Northern blot analysis*

A 2-μg portion of poly(A^+) RNA from each tissue was electrophoresed on a 1% agarose–2.2 M formaldehyde gel. A blotted membrane was hybridized with a ^{32}P-labelled insert of the RT-PCR clone PTE45 using a rapid hybridization buffer (Amersham). A mixture of ^{32}P-labelled inserts of five PTE clones excluding PTE45 was also used in a case. The filter was washed at 65°C in 0.5 × SSC containing 0.1% SDS.

3. RESULTS AND DISCUSSION

To identify G protein-coupled receptors in lingual epithelia, several oligonucleotide primers corresponding to amino acid sequences of olfactory receptor molecules [8] around transmembrane domains II, VI and VII, were prepared and subjected to a reverse transcription-polymerase chain reaction (RT-PCR) using mRNA obtained from apical epithelial cells of a rat tongue tip. A mini-library of RT-PCR products with ca. 500 bp each in length was screened with an internal oligonucleotide probe corresponding to transmembrane

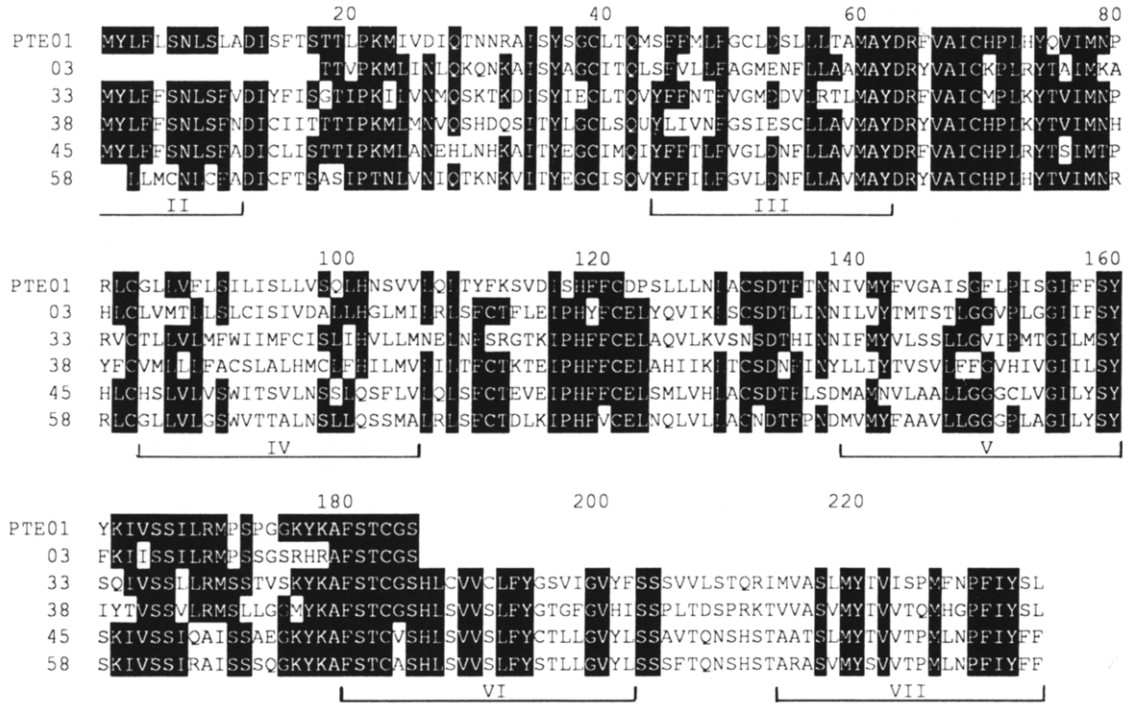

Fig. 1. Alignment of deduced amino acid sequences of six RT-PCR clones encoding putative G protein-coupled receptors in lingual epithelia. Consensus sequence matches, i.e. at least four out of the six sequences, are denoted by white letters in black boxes. The inferred positions of putative seven-transmembrane domains are underlined.

domain III generally conserved in the G protein-coupled receptor superfamily [16]. Nucleotide sequencing of the positive clones revealed more than 60 clones encoding closely related but partly different proteins, all of which contained several transmembrane domain motifs commonly observed in the various G protein-coupled transmembrane receptors [16]. By comparison of the deduced amino acid sequences, the 60 clones were classified into six distinct groups, each comprising three to fifteen clones. The deduced amino acid sequences of the representatives of the six groups are shown in Fig. 1. The sequences of the RT-PCR clones of lingual origin, though lacking N- and C-terminal regions, show a typical feature common among other receptors in sensory systems such as olfactory receptors [8] and rhodopsin [17]. Thus, a large number of G protein-coupled

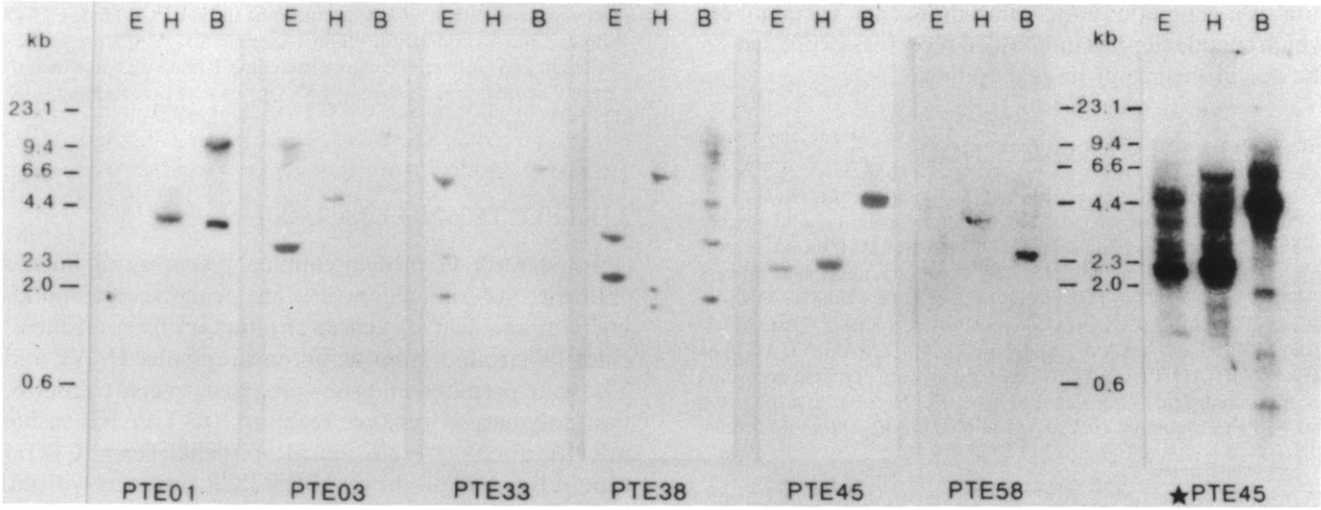

Fig. 2. Identification of the genes for putative lingual receptors of rat. Genomic DNA was isolated from rat liver and digested with *Eco*RI (E), *Hin*dIII (H) and *Bam*HI (B) and subjected to Southern blot hybridization using RT-PCR clones PTE 01, 03, 33, 38, 45 and 58. Right panel (*PTE45) represents a result from an experiment carried out under a low stringency conditions of washing.

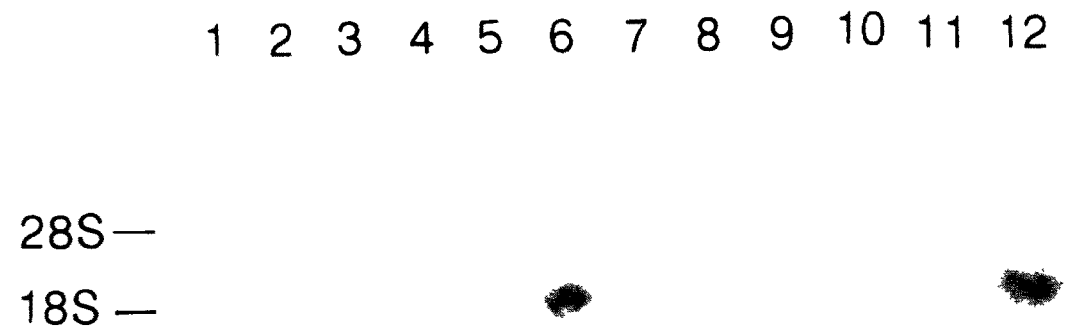

Fig. 3. Expression of the clone PTE45 in various rat tissues. The cDNA insert of PTE45 encoding one of putative lingual receptors was used as a probe for Northern blot analysis. Migration of 28 S and 18 S RNA is shown at the right. Lane 1, liver; lane 2, kidney; lane 3, testis; lane 4, brain; lane 5, tongue total; lane 6, tongue epithelium; lane 7, retine; lane 8, lung; lane 9, small intestine; lane 10, skeletal muscle and lane 11, smooth muscle. Lane 12 shows a result of the hybridization of a tongue epithelium hybridized with a mixture of five PTE clones excluding the PTE 45 clone.

receptors with similar structural characteristics are thought to be expressed in the epithelial cells of tongue.

To obtain further information about the gene family, genomic Southern analyses were conducted with the six RT-PCR clones (Fig. 1) as probes. Under stringent conditions for hybridization, each of the DNA probes gave a simple pattern, indicating that the genes are essentially single copy genes (Fig. 2). However, under reduced stringency conditions, a cDNA insert of PTE45 hybridized several bands with various intensities (Fig. 2, *right*), showing that genes for lingual epithelium receptors constitute a large gene family, probably made up out of more than 60 members, as suggested from the number of different RT-PCR clones. From these molecular studies, a large gene family of G protein-coupled receptors is suggested to be possibly involved in transmembrane signalling for taste stimulus transduction in lingual epithelia.

We next examined whether the expression of these receptors is tongue-specific. The result of the Northern blot analysis cleary demonstrates that PTE45 mRNA is specifically expressed in epithelial cells of the tongue as a single species of about 2 kb in length (Fig. 3, lane 6); no expression was detected in other organs. Also, the signal was scarcely detected when whole tongue mRNA was used (Fig. 3, lane 5), probably indicating that the expression is restricted to the epithelium. Similar results were obtained when a mixture of five PTE clones excluding PTE45 clone was used for the Northern blotting. The mixed probe hybridized one broad band of about 2 kb among tongue epithelium mRNA species (Fig. 3, lane 12).

We have shown here that the multiple G protein-coupled receptor genes, closely related to each other in terms of structure, are expressed in the epithelial cells. These receptors may be involved in the taste-signalling that starts with transmembrane events connected to intracellular signalling, leading to neural signalling of the neighbouring axons of sensory neurons. In such intracellular signalling, taste transduction is characterized by three events according to the type of taste stimuli: changes in ion channel-gating by salty and sour tastes [18,19], cAMP concentration (enhancement) by sweet and bitter tastes [12,20], and Ca^{2+} influx by bitter taste [21]. At least, the latter two may be mediated by transmembrane G protein-coupled receptors located on the taste cell surface, to which the newly identified receptors described here might be related. The possibility thus exists that these lingual epithelium receptors may function by coupling with α-gustducin reported to be a novel taste-cell-specific Gα protein possibly involved in taste stimulus transduction [14]. There is also another possibility that the lingual receptors may couple with the sweet- taste-specific G_s protein the occurrence of which has been predicted by biochemical studies using a rat tongue-tip-rich in fungiform papillae [12]. Taken together, the multiple species of seven-transmembrane proteins identified here could be potent candidates for the taste signal receptors. Further cellular and molecular analyses will provide more direct evidence towards understanding the entire molecular mechanism of taste transduction.

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