Cloning and Characterization of a Novel mGluR1 Variant from Vallate Papillae that Functions as a Receptor for L-glutamate Stimuli

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Introduction

Monosodium L-glutamate (MSG) functions as a signal for dietary protein. In the tongue, glutamate binds presumably to taste cell chemoreceptors which, upon being activated, alter the firing rate of innervating sensory nerves. It has just recently been hypothesized that several G protein coupled receptors (GPCR) acted as umami receptors (Chaudhari et al., 2000; Li et al., 2002; Nelson et al., 2002; Toyono et al., 2003). However, the receptor mechanism for umami taste perception is still in doubt (Damak et al., 2003; Zhao et al., 2003). The data from gustatory nerve recordings, receptor distribution and taste cell electrophysiological function cannot all be reconciled solely by reference to already known receptors (Hoon et al., 1999; Ninomiya et al., 2000; Kim et al., 2003). Furthermore, since knocking out T1r3 does not affect umami responses originating from the back of the tongue, other receptors must be considered (Damak et al., 2003). In this study, we describe a novel metabotropic glutamate receptor 1 (mGluR1) variant. This variant was cloned from rat vallate tissue and has a unique 5′ end sequence. In-frame with the long open reading frame there is a stop codon suggesting the presence of an un-translated 5′ region producing a short extracellular domain. Truncated mGluR1 generated intracellular Ca2+-dependent Cl− current responses in Xenopus oocytes when L-glutamate was applied at concentrations that elicit the umami taste.

Materials and methods

Vallate and foliate papillae and soft palate epithelium were dissected from adult Sprague-Dawley rats (Charles River, Japan). Tissue total RNA was then extracted with ISOGEN kit (Wako, Osaka, Japan) and first-strand 5′ RACE (rapid amplification of cDNA ends) reaction (SMART RACE cDNA amplification kit; Clontech Laboratories, USA). A rat mGluR1-1599R gene-specific primer (5′-CTGTCTCT-GGACATAGTTTTCTTC-3′) was synthesized at Hokkaido System Science (Hokkaido, Japan). After sequence analysis, full-length cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA). Using the clone as template, cDNA was produced by PCR with Pfu DNA polymerase enzyme (Promega, USA) and cloned into pcDNA3.1/V5-His vector (TOPO TA Expression Kit; Invitrogen, USA).

Results

The 5′ RACE reaction resulted in a short PCR product of around 400 bp. Sequence analysis revealed a 5′ end consisting of the last 170 nucleotides from the preceding intron to codon D318 (GRM1, accession No. NW_047544). The intron sequence revealed a stop codon in-frame with the long open reading frame that shares high homology with brain mGluR1a. Downstream of the exon sequence there is a putative start codon, M410. The existence of the stop codon suggests that the 5′ end is not translated and tentatively yields a short receptor.

The function of the truncated taste type receptor was examined by comparing its activity with that of the brain mGluR1t6 in Xenopus oocytes. As shown in Figure 1, several functional differences are noted between the brain type mGluR1, with a long N-terminus, and the taste variant, with a shorter extracellular domain. Upon activation with L-glutamate, the brain type elicited a maximal downward current nine times greater on average than that elicited by taste mGluR1. In addition, taste variant mGluR1 responded to L-glutamate stimuli at concentrations that normally elicit umami taste in rat. Different concentrations of L-glutamate evoked distinctive current responses in brain and taste type mGluR1. While the brain type receptor achieved maximal response at 1 mM L-glutamate, the taste type mGluR1 responded best to higher L-glutamate concentrations indicating a lower sensitivity to glutamate.

Discussion

Our data show that the mGluR1t6 expressed in taste buds is structurally distinct from the one expressed in brain. Like the structure reported for taste-mGluR4 (Chaudhari et al., 2000), the mGluR1 cloned from taste papillae also contains a short amino-terminal extracellular domain. Unlike mGluR4, however, which is an inhibitory receptor, mGluR1 is an excitatory receptor. The mGluR1 taste variant is probably synthesized from a splice transcript with a short 5′ sequence or by the activation of an ectopic promoter in the preceding intron (Yamaguchi and Nakashiba, 1998). Despite the unusually short extracellular amino-terminal region, taste variant mGluR1 was able to elicit glutamate-stimulated functional changes in Xenopus oocytes. Metabotropic glutamate receptors contain a characteristic long extracellular domain that apparently functions in determining the affinity of the receptor for L-glutamate (Takahashi et al., 1993). In agreement with this suggestion, the truncated taste mGluR1 showed a lower sensitivity to glutamate than the brain mGluR1. The L-glutamate concentration that brought about maximum current amplitude in Xenopus oocytes expressing the taste type mGluR1 was in the millimolar range. This concentration is consistent with the amount of glutamate necessary to elicit gustatory
stimulation. Histological, electrophysiological and molecular data together suggest that taste variant mGluR1 is involved in L-glutamate perception. As the prototypical umami stimulus, and a likely taste marker for protein, L-glutamate may both stimulate a taste sensation and also confer information as to the probable chemical composition of ingested foodstuffs. This information on the chemical identity of food allows brain relays to anticipate the arrival of specific foods and thereby insure efficient digestion and metabolism.

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References


Figure 1 Glutamate activates the truncated taste-mGluR1 to produce an inward current. Gustatory mGluR1 has functional characteristics in Xenopus oocytes different from those of brain mGluR1. Taste type mGluR1 stimulates maximal inward current at a higher l-glutamate concentration (25 mM) than that compared with the brain type (0.1 mM). Schemes represent brain type (left) and taste type (right) mGluR1 with their typical current traces. This experiment was performed on control oocytes or oocytes injected with either brain or taste mGluR1 cRNA and analyzed under –70 mV voltage clamp. Different concentrations of l-glutamate were constantly perfused for 30 s into the recording chamber as stimuli for each receptor as indicated with the arrows. Four to six different oocytes were recorded for brain and taste type mGluR1 respectively. Downward curve represents an inward current as a result of increased Cl− conductance due to intracellular Ca2+ mobilization. Intracellular injection of EGTA in brain type mGluR1 expressing oocytes effectively suppresses glutamate response of mGluR but not a voltage-dependent potassium channel (Masu et al., 1991). This selective inhibition of mGluR1 activation by intracellular Ca2+ chelation demonstrates that Ca2+ mobilization is a necessary step to induce inward currents after receptor stimulation. The absence of external Ca2+ in the medium doesn’t affect mGluR1 stimulation response. Scale bars: vertical 500 nA (brain) 100 nA (taste); horizontal, 20 s.