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 bud 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). The glossohygreal nerve that innervates foliate and vallate papillae at the posterior tongue contains fibers that are highly sensitive and selective to umami substances. Such umami-sensitive fibers were not observed in the chorda tympani nerve innervating fungiform papillae of anterior tongue (Ninomiya et al., 2000). However, the proposed amino-acid receptor T1R1 + T1R3 seems to be mostly abundant in fungiform papillae (Hoon et al., 1999; 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+ mobilization in Xenopus oocytes when L-glutamate was applied at concentrations that elicited 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). 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). After sequencing, the PCR product was synthesized using SuperScript reverse transcriptase, oligo(dT)12-18 primer (both from Invitrogen, USA) and SMART II oligonucleotide (SMART RACE cDNA amplification kit; Clontech Laboratories, USA). A rat mGluR1-159R gene-specific primer (5′-CGTGCTCT-GGACATAGTTTCTTC-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 synthesized using SuperScript reverse transcriptase, oligo(dT)12-18 primer (both from Invitrogen, USA) and SMART II oligonucleotide (SMART RACE cDNA amplification kit; Clontech Laboratories, USA). A rat mGluR1-159R gene-specific primer (5′-CGTGCTCT-GGACATAGTTTCTTC-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 generated with a T7 transcription kit (mMessage mMACHINE; Ambion, USA). Xenopus oocytes (Watanabe Breeder, Japan) retaining clear animal and vegetal pole were injected (micro-injector; WPI) with 100 ng taste- and brain-mGluR1 cRNA. After characterization of a novel mGluR1 variant from vallate papillae that functions as a receptor for L-glutamate stimuli.
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


