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Serotonin differentially modulates the electrical properties of different subsets of taste receptor cells in bullfrog

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Abstract

Serotonin (5-hydroxytryptamin, 5-HT) is localized in taste bud cells of vertebrates. Effects of the external application of 5-HT on the membrane currents of frog taste receptor cells (TRCs) were investigated using patch-clamp technique in whole-cell configuration. The 5-HT (0.1–1 μM) and 5-HT_{1A} receptor agonist (+/–)-8-OH-2-(D1-*n*-propyl-amino)tetralin (8-OH-DPAT) (1–20 μM) inhibited both voltage-gated sodium current (I_{Na}) and voltage-gated potassium current (I_K) in 50% of TRCs, but potentiated I_K without any significant effect on I_{Na} in another subset of 18% of TRCs. Voltage-gated currents in the residual TRCs were not affected by 5-HT or 8-OH-DPAT. External application of 10 μM forskolin and 300 μM 8-cpt cAMP [8-(4-chlorophenylthio)adenosine 3′:5′-cyclic monophosphate] mimicked the inhibitory effect of 5-HT and 8-OH-DPAT on I_K and I_{Na} while internal dialysis with 50 μM protein kinase A inhibitor prevented the 5-HT-mediated inhibitory effects on I_K and I_{Na} in TRCs. Internal dialysis of TRCs with high Ca^{2+}-pipette solution (1 μM) increased the I_K in 58% of TRCs. The 5-HT reversibly increased the $[Ca^{2+}]_i$ in 17% of TRCs when measured by Ca^{2+}-imaging using a Ca^{2+}-sensitive dye (fura-2 AM). These results suggest that 5-HT differentially modulates the voltage-gated membrane currents in different subsets of TRCs.

Introduction

Taste receptor cells (TRCs) detect the gustatory information. Information of taste stimulus is converted into electrical signals by the TRCs and carried away in the facial, glossopharyngeal and vagus nerves. At receptor level, neuroactive substances, such as serotonin (5-HT), can modulate either the synaptic transmission at the synaptic cleft or the excitability of TRCs by modulating the ion channels through second messenger systems. Immunochemical and histochemical studies suggest that serotonin is present in taste bud cells of a variety of vertebrates (Fujimoto et al., 1987; Kim & Roper, 1995). In frog and mudpuppy, the presence of 5-HT has been demonstrated in Merkel-like basal cells of the taste buds (Delay et al., 1993; Lindemann, 1996; Nagai et al., 1996). In the mudpuppy, bath application of 5-HT produced a hyperpolarization of resting potential, an increase in input resistance and the amplitude of KCl-induced receptor potential of TRCs. The same effects were observed when basal cells were repetitively stimulated during recording from receptor cells, suggesting that 5-HT is released from basal cells (Ewald & Roper, 1994). It has been reported that 5-HT injected into the lingual artery suppressed the gustatory neural responses for KCl in frogs (Morimoto & Sato, 1977). However, subepithelial injection of 5-HT into the tongue increased both the spontaneous activity and the responses for basic taste stimuli of glossopharyngeal nerve (Esakov et al., 1983). Differential modulation of voltage-gated calcium current (I_{Ca}) by 5-HT in *Necturus* TRCs has been reported. Application of 100 μM 5-HT increased I_{Ca} in 33% of TRCs, whereas it decreased I_{Ca} in 67% of TRCs (Delay et al., 1997). These effects have been attributed to the 5-HT_1 and 5-HT_3 type of receptor mechanisms. We have also reported that 5-HT inhibits voltage-gated sodium current (I_{Na}) (Imendra et al., 2000).

In this study, we have further investigated the effect of 5-HT and serotonin receptor subtype 1A (5-HT_{1A}) agonist (+/–)-8-OH-2-(D1-*n*-propyl-amino)tetralin (8-OH-DPAT) on (i) modulation of I_{Na} and voltage-gated potassium current (I_K); (ii) excitability of TRCs under current-clamp mode; and (iii) changes of internal calcium concentration ($[Ca^{2+}]_i$) by a Ca^{2+}-imaging technique. We found that frog TRCs can be categorized into three different functional subsets by the responses to 5-HT and 8-OH-DPAT.

A preliminary report of some of these results has been published in abstract form (Imendra et al., 2001).

Materials and methods

Preparation of TRC

Bullfrogs (*Rana catesbeiana*) weighing 250–550 g were used. The experiments were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University. TRCs were isolated from the tongue of decapitated and pithed animals, as described previously (Miyamoto et al., 1991; Okada et al., 1996). Briefly, the fungiform papillae were dissected out from isolated tongues and collected into a nominally Ca^{2+}-free saline solution. The papillae were treated with papain (8.4 U/mL, Sigma, St Louis, MO, USA) activated by L-cysteine (10 mM, Sigma) in 2 mL divalent cation-free saline solution containing 2 mM EDTA for 10 min. After the treatment, papillae were washed with normal Ringer solution (NRS) to remove the enzyme and gently triturated to dissociate the TRCs. Dissociated cells were filtered across a 100-μm^2 nylon mesh and stored in ice-cold NRS.

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