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Research Report

5-HT$_3$ receptor expression in the mouse vestibular ganglion

Yasumitsu Takimoto[a,b], Yusuke Ishida[b,*], Yukiko Nakamura[b], Takefumi Kamakura[a,b], Takahiro Yamada[b], Makoto Kondo[b], Tadashi Kitahara[a], Atsuhiko Uno[a], Takao Imai[a], Arata Horii[a], Suzuyo Okazaki[a], Suetaka Nishiike[a], Hidenori Inohara[a], Shoichi Shimada[b]

[a]Department of Otorhinolaryngology – Head and Neck Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

[b]Department of Neuroscience and Cell Biology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

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ABSTRACT

The 5-hydroxytryptamine type 3 (5-HT$_3$) receptor is a ligand-gated ion channel and a member of the Cys-loop family of receptors. Previous studies have shown 5-HT$_3$ receptor expression in various neural cells of the central and peripheral nervous systems. Although the function and distribution of the 5-HT$_3$ receptor has been well established, its role in the inner ear is still poorly understood. Moreover, no study has yet determined its localization and function in the peripheral vestibular nervous system. In the present study, we reveal mRNA expression of both 5-HT$_{3A}$ and 5-HT$_{3B}$ receptor subunits in the mouse vestibular ganglion (VG) by RT-PCR and *in situ* hybridization (ISH). We also show by ISH that 5-HT$_3$ receptor mRNA is only expressed in the VG (superior and inferior division) in the peripheral vestibular nervous system. Moreover, we performed Ca^{2+} imaging to determine whether functional 5-HT$_3$ receptors are present in the mouse VG, using a selective 5-HT$_3$ receptor agonist, SR57227A. In wild mice, 32% of VG neurons responded to the agonist, whereas there was no response in 5-HT$_{3A}$ receptor knockout mice. These results indicate that VG cells express functional 5-HT$_3$ receptor channels and might play a modulatory role in the peripheral vestibular nervous system.

1. Introduction

In contrast to the other 5-hydroxytryptamine (5-HT) receptor subfamilies, that are all G protein coupled receptors, the 5-HT type 3 (5-HT$_3$) receptor is the only ligand-gated cation channel. It is a member of the Cys-loop family of receptors, which also includes the glycine, GABA$_A$ and nicotinic acetylcholine receptors, and plays major roles in fast synaptic transmission (Derkach et al., 1989; Lester et al., 2004; Maricq et al., 1991; Yakel and Jackson, 1988). In rodents, the 5-HT$_3$ receptor consists

*Corresponding author. Fax: +81 6 6879 3129.
E-mail address: ishida@anat1.med.osaka-u.ac.jp (Y. Ishida).

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of two subunits (A and B); however, the human genome contains five genes encoding different 5-HT_3 subunits (A, B, C, D and E). The 5-HT_{3A} and 5-HT_{3B} receptor subunits are mainly involved in formation of functional receptors. Both 5-HT_{3A} homomeric and 5-HT_{3AB} heteromeric receptors can form functional receptors, whereas 5-HT_{3B} homomeric receptors are not functional. 5-HT_{3AB} heteromeric receptors have different pharmacological and biophysical properties from 5-HT_{3A} homomeric receptors, exhibiting large single-channel conductance, low permeability to calcium ions, and a linear current-voltage relationship (Davies et al., 1999; Niesler et al., 2003, 2007).

Previous studies have shown 5-HT_3 receptor expression in various neurons of the central nervous systems (CNS), and its involvement in anxiolytic action and cognitive functions, such as learning, attention, memory and fear extinction (Bhatnagar et al., 2004; Harrell and Allan, 2003; Kelley et al., 2003; Kondo at el., in press; Miquel et al., 2002; Morales et al., 1996, 1998; Tecott et al., 1993; Thompson and Lummis, 2007). In the peripheral nervous system, 5-HT_3 receptor expression has been found in dorsal root, nodose, superior cervical, trigeminal, and vagal nerve ganglia (Hoyer et al., 1989; Rosenberg et al., 1997; Morales et al., 2001; Morales and Wang, 2002), with functional involvement in the vomiting reflex, tissue injury-induced pain, and hyperalgesia (Galligan, 2002; Liang et al., 2011; Minami et al., 2003, Sommer, 2004; Zeitz et al., 2002). Generally, although the function and distribution of 5-HT_3 receptors is well established, its role in the inner ear is still not understood. To our knowledge, no study has been done on the expression and function of 5-HT_3 receptor in the peripheral vestibular nervous system. A few studies have reported the expression of 5-HT receptors in the inner ear. Expression of 5-HT receptors, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5B} and 5-HT_6 receptor subunits, have been shown in mouse cochlear tissues by Reverse-transcription polymerase chain reaction (RT-PCR) (Oh et al., 1999). In addition, the distribution of 5-HT_{1B} and 5-HT_{1D} receptor subunits in rat and monkey inner ear has been shown by immunohistochemistry, and suggests that the receptors contribute to comorbidity of headache and vertigo in migraine (Ahn and Balaban, 2010). Nevertheless, the role of 5-HT receptors is still unclear in the inner ear.

Interestingly, a recent clinical study reported the preventive effect of ondansetron, a 5-HT_3 receptor antagonist, on the vestibular deficit in acute-phase vestibular neuritis (Venail et al., 2012). Although the basis of this mechanism is unknown, it has been speculated that the 5-HT_3 receptor plays a functional role in the vestibular system. Therefore, to address this question, we investigate here the localization of the 5-HT_3 receptor in the mouse peripheral vestibular nervous system.

2. Results

2.1. 5-HT_{3A} and 5-HT_{3B} receptor subunit mRNA expression in the mouse vestibular ganglion

2.1.1. Reverse transcription-polymerase chain reaction (RT-PCR)

We performed RT-PCR analysis on adult mouse whole inner ear, VG, and whole cochlea tissues (Fig. 1). First, we observed that both 5-HT_{3A} (HTR3A) and 5-HT_{3B} receptor (HTR3B) transcripts were found in the whole inner ear tissue (Fig. 1A). Next, we closely examined 5-HT_3 receptor expression in each inner ear division. HTR3A and HTR3B transcripts were both detected in the VG, while only 5-HT_{3A} receptors were detected in the cochlea tissue (Fig. 1B, upper panel). The trigeminal ganglion (TG) was shown as a positive control for HTR3A and HTR3B expression (Fig. 1B, bottom panel; Morales and Wang, 2002). Control β-actin fragments are also shown in Fig. 1. These results suggest that 5-HT_{3A} receptors are expressed in the VG and cochlea, while 5-HT_{3B} receptors are only expressed in the VG. No (or very weak) 5-HT_{3B} receptor signal was detected in the cochlea.

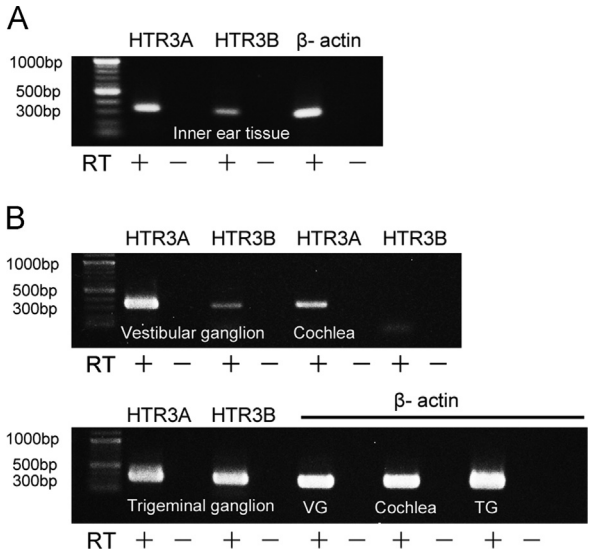

Fig. 1 – 5-HT_3 expression in adult mouse vestibular ganglion, revealed by RT-PCR. Both 5-HT_{3A} (HTR3A) and 5-HT_{3B} (HTR3B) receptor signals were detected in whole inner ear tissue (A) and vestibular ganglion (VG), while only HTR3A signal was detected in cochlea tissue (B, upper panel).The trigeminal ganglion (TG) is shown as a positive control for HTR3A and HTR3B expression (B, bottom panel). Control β-actin transcripts are shown at the right-hand side of the bottom panel. RT (−) indicates template cDNA without reverse transcriptase. The DNA standard is shown on the left-hand side of each panel.

2.1.2. In situ hybridization

To further evaluate the RT-PCR findings, we performed ISH using digoxigenin (DIG)-labeled cRNA probes in the inner ear. We used two different chromogenic reaction protocols: green (Alexa 488) and red (Fast Red). The green protocol had higher sensitivity and intensity but amplified mRNA signals resulted in a high level of noise. As such, it was suitable for low-power viewing or amplification of weakly expressed genes. The red protocol was suitable for high-power viewing.

First, we examined 5-HT_3 receptor mRNA localization in the inner ear (Fig. 2). We used postnatal day (P) 5 tissue, which required no decalcification. For the purpose of examining the anatomy of the inner ear, we chose Alexa 488 as the fluorophore (shown in green). Because 5-HT_{3B} receptor (HTR3B) mRNA hybridization signals were very weak (Fig. 2I), we chose to show only 5-HT_{3A} receptor mRNA localization anatomically.