Brain Research

Research Report

Mu-opioid receptor (MOR) expression in the human spiral ganglia

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ABSTRACT

Opioid peptides and their receptors have been localized to the inner ear of the rat and guinea pig mammalian models. The expression of mu opioid receptor (MOR) in the human and mouse cochlea is not yet known. We present MOR protein localization by immunohistochemistry and mRNA expression by *in situ* hybridization in the human and mouse spiral ganglia (SG) and organ of Corti. In the human most of the (SG) neurons were immunoreactive; a subset was non-immunoreactive. *In situ* hybridization revealed a similar labeling pattern across the neurons of the SG. A similar distribution MOR pattern was demonstrated in the mouse SG. In the mouse organ of Corti MOR was expressed in inner and outer hair cells. Fibers underneath the inner hair cells were also MOR immunoreactive. These results are consistent with a role of MOR in neuromodulation of the auditory periphery. The present results show that the expression of MORs is well-conserved across multiple mammalian species, indicative of an important role in auditory processing.

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1. Introduction

The opioid peptides are a family of over twenty endogenous neuromodulators that are produced by neurons throughout the mammalian central nervous system. They bind to and activate seven transmembrane guanine nucleotide-binding protein coupled opioid receptors (Waldhoer et al., 2004; Zuo, 2005). Opioid receptors are located through the body, and regulate a number of important behaviors such as reward, pain, stress, gastrointestinal transport and mood through receptors in both the central and peripheral nervous system (Pradhan et al., 2012). There are four types of opioid receptors—mu-opioid receptor (MOR), kappa-opioid receptor (KOR), delta-opioid receptor (DOR), and nociceptin opioid

receptor (NOP) (McDonald and Lambert, 2005; Waldhoer et al., 2004). MOR is involved in respiration, cardiovascular function, thermoregulation, immune function, and gastrointestinal motility, along with the mediation of common analgesia and euphoria pathways (Contet et al., 2004; McDonald and Lambert, 2005). Knockout studies in mice show that the MOR is essential for morphine-induced analgesia, genetic variation of the MOR genre (OPRM1) has been associated with variation in opioid response in different setting including acute post-operative pain, chronic non-cancer pain and cancer related pain (revised by Branford et al., 2012).

In addition to binding endogenous opioid peptides, opioid receptors are also the target of analgesic and psychoactive opioid agents that are often prescribed for analgesia, as well as commonly used for recreational purposes. Frequently-used opioids include morphine, codeine, hydrocodone, her-oine, and cocaine. Studies have shown that a staggering 90% of patients with chronic pain rely on opioid medications for analgesia, and additionally 5.6% of the adult population uses opioids for non-medicinal purposes (United Nations, 2011).

Well-known side effects of opioids include dizziness, nau-sea, vomiting, respiratory depression, and death in overdose (Trescot et al., 2008, 2010). A less well-known adverse effect of opioids is sudden or rapidly-progressive sensorineural hearing loss. Multiple reports have linked hydrocodone/acetaminophen, oxycodone/acetaminophen, propoxyphene, heroin, cocaine, methadone, and amphetamine use with severe to profound sensorineural hearing loss (Christenson and Marjala, 2010; Ciorba et al., 2009; Fowler and King, 2008; Friedman et al., 2000; Harell et al., 1978; Ho et al., 2007; Iqbal, 2004; Ishiyama et al., 2001; Oh et al., 2000; Rigby and Parnes, 2008; Schrock et al., 2008; Stenner et al., 2009; van Gaalen et al., 2009). Some of these studies reported individuals that used legally-prescribed med-ications, such as Vicodin, not always at high doses, but some-times within the recommended dosages. In some cases, the bilateral hearing loss is irreversible, and cochlear implantation restores functional hearing (Freeman et al., 2009; Friedman et al., 2000; Ho et al., 2007; Oh et al., 2000).

Opioid neuropeptides (enkephalins) and their receptors are expressed in both the central and peripheral auditory system. Fex and Altschuler (1981) demonstrated the existence of encephalin-like immunoreactivity in the organ of Corti of the guinea pig and cat. Studies on the distribution of opioid receptors in the auditory and vestibular periphery have been conducted also in the rat and guinea pig cochlea (Jongkamonwiwat et al., 2003, 2006) using immunocytochemistry, western blot and RT-PCR. All four opioid receptor subtypes (MOR, DOR, KOR and ORL) were detected. Prior studies have localized MOR to the auditory and vestibular systems of several mammalian and amphibian species. Specifically, MOR has been found in the supporting cells and spiral ganglia of the rat (Jongkamonwiwat et al., 2003), as well as in the inner and outer hair cells, Deiter's cells, and the inner and outer spiral bundles of the guinea pig (Jongkamonwiwat et al., 2006). In the rat vestibular periphery, MOR mRNA and protein expression has been discovered in the calyceal and bouton afferents (Popper et al., 2004).

The functionality of opioid receptors in the inner ear has been previously investigated (Vega and Soto 2003, Lioudyno et al., 2000). Opioid drugs effects on auditory evoked poten-tials suggest a role of lateral olivocochlear dynorphins in auditory function (Sahley et al., 1991); opioid receptors inhibit the adenylate cyclase in guinea pig cochleas (Eybalin et al. 1987); opioid receptors mediate a postsynaptic facilitation and a presynaptic inhibition at the afferent synapse if axolotl vestibular hair cells (Vega and Soto, 2003); the alpha9/alpha10-containing nicotinic Ach receptor is directly modu-lated by opioid peptides (Lioudyno et al., 2000). This type of opposed pre- and postsynaptic action is typical of opioid receptors and accounts for the state-dependent activity of peptide neuromodulators (Soto and Vega, 2010).

Despite multiple studies demonstrating the presence of MOR in the inner ear of various animal models, MOR has yet to be localized to either the human or mouse cochlea. In the present study using immunocytochemistry and *in situ* hybri-dization we study the expression of MOR in the human and mouse auditory periphery. We found a similar expression of MOR in the cochlea of both species.

2. Results

MOR Immunoreactivity (-IR) in the human SGNs. MOR-IR was visualized by indirect immunohistochemistry using horse-radish peroxidase and the chromogen diaminobenzidine (Fig. 1). Immunoreactivity was confined to neuronal somata, nuclei was non-immunoreactive. Moderated immunoreactiv-ity was seen in nerve fibers than intermingle with the neurons in the SG. Satellite cells that surround SGNs were not immunoreactive to MOR antibodies. MOR-IR was present in most SGNs at the apical (Fig. 1a), middle (Fig. 1b) and basal (Fig. 1c), levels of the cochlea. The pattern of immunoreac-tivity was similar in the three levels, and consistent in all specimens immunoreacted with MOR antibody. Fig. 1d, shows a negative control; MOR antibody was omitted in the immunoreaction, and SGNs were not immunostained.

Distribution of MOR-mRNA-positive cells in the human SGNs. A digoxigenin-labeled antisense oligonucleotide probe for the human and mouse MOR was used to detect the expression of MOR in the human and mouse SGNs. MOR-mRNA positive neurons were detected in the SGNs. The expression pattern detected by *in situ* hybridization was similar to the one obtained by immunocytochemistry. Fig. 2a and b shows SGNs from two regions of the cochlea (base and middle portion). Fig. 2c shows a high magnification view. MOR-mRNA is present in most of the SGNs somata. The staining pattern was similar in the neurons of the human SG from the base to the apical portion. Consistent positive signal was seem among the specimens stained.

MOR-IR and mRNA in the mouse SGNs. In the mouse SG, MOR-IR was present in the soma of the SGNs, their nuclei were non-reactive. Neurons were immunoreactive to the MOR antibody at the apical (Fig. 3a), middle (Fig. 3a′), and basal (Fig. 3a″) levels of the SG; however, some neurons showed stronger signal. Immunoreactive and non immunor-eactive neurons were of similar size. Fig. 3b is a negative control, primary antibody was omitted no immunoreaction was observed. MOR-mRNA signal pattern was similar to the immunoreactive pattern; most of the neurons showed MOR-mRNA positive signal (Fig. 3c). Fig. 3c′ shows SGNs hybridized with sense strands, in which no signal was detected.