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Research paper

Expression patterns of atrial natriuretic peptide and its receptors within the cochlear spiral ganglion of the postnatal rat

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ABSTRACT

The spiral ganglion, which is primarily composed of spiral ganglion neurons and satellite glial cells, transmits auditory information from sensory hair cells to the central nervous system. Atrial natriuretic peptide (ANP), acting through specific receptors, is a regulatory peptide required for a variety of cardiac, neuronal and glial functions. Although previous studies have provided direct evidence for the presence of ANP and its functional receptors (NPR-A and NPR-C) in the inner ear, their presence within the cochlear spiral ganglion and their regulatory roles during auditory neurotransmission and development is not known. Here we investigated the expression patterns and levels of ANP and its receptors within the cochlear spiral ganglion of the postnatal rat using immunofluorescence and immunoelectron microscopy techniques, reverse transcription-polymerase chain reaction and Western blot analysis. We have demonstrated that ANP and its receptors colocalize in both subtypes of spiral ganglion neurons and in perineuronal satellite glial cells. Furthermore, we have analyzed differential expression levels associated with both mRNA and protein of ANP and its receptors within the rat spiral ganglion during postnatal development. Collectively, our research provides direct evidence for the presence and synthesis of ANP and its receptors in both neuronal and non-neuronal cells within the cochlear spiral ganglion, suggesting possible roles for ANP in modulating neuronal and glial functions, as well as neuron–satellite glial cell communication, within the spiral ganglion during auditory neurotransmission and development.

1. Introduction

The spiral ganglion is a peripheral cluster of both neurons and glial cells localized in Rosenthal's canal, which coils around the cochlear modiolus. Spiral ganglion neurons (SGNs) are the primary afferent neurons in the spiral ganglion and play a critical role in hearing, transmitting primary acoustic information from the mechanosensory hair cells in the organ of Corti to the higher auditory centers of the central nervous system (CNS) (Nayagam et al., 2011; Rusznák and Szűcs, 2009). SGNs are divided into two subpopulations, type I and type II, according to their different morphologies, synaptic connections and functions. The large, bipolar and myelinated type I neurons innervate the inner hair cells with their peripheral processes to principally encode the auditory signals and represent approximately 90–95% of the afferent auditory neurons. The smaller, pseudomonopolar and non-myelinated type II neurons innervate the outer hair cells and some of the cochlear supporting cells to provide sensory feedback, controlling the sensitivity of the auditory epithelia to specific sound stimuli. Degeneration of SGNs, primarily resulting from noise trauma, ototoxic drugs, infection, aging and genetic mutations, or secondarily occurring as a result of hair cells loss, ultimately leads to permanent sensorineural hearing loss (Shepherd and Hardie, 2001). Understanding the expression, function and signaling interactions of the neurotransmitters, neuromodulators and other regulatory substances which affect neuronal physiology and neurotransmission in primary auditory neurons, may offer insights

Abbreviations: ANP, atrial natriuretic peptide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; GC, guanylyl cyclase; NPR-A, natriuretic peptide receptor-A; NPR-C, natriuretic peptide receptor-C; RT-PCR, reverse transcription-polymerase chain reaction; SGCs, satellite glial cells; SGNs, spiral ganglion neurons

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into the mechanisms underlying normal and pathological states of hearing, and provide important clues for effective prophylactic and therapeutic treatment for hearing impairment.

The satellite glial cells (SGCs) and Schwann cells are the primary glial cells in the spiral ganglion. Schwann cells are found in close proximity to the neuronal processes or axons where they form myelin sheaths around type I neuronal axons. Perineuronal SGCs, whose roles are still relatively poorly understood, envelop the somata of SGNs, and together they constitute functional units. Additionally, SGCs form loose myelin around the somatic membrane of type I neurons. SGCs in the peripheral sensory ganglion share many properties with astrocytes in the CNS, as they not only express various ion channels, neurotransmitter receptors and trophic factors which modulate neuronal activity in synaptic transmission, but also form a partial diffusion barrier which regulates the neuronal environment, resembling the blood–brain barrier in the CNS (Barres, 2008; Hanani, 2010). Consequently, the interaction between neurons and SGCs can modulate neurotransmission, neuroprotection and neuronal homeostasis (Hanani, 2005). Therefore, new strategies aimed at manipulating communication between SGNs and SGCs within the spiral ganglion have the potential to become promising pharmacological targets that facilitate new and effective therapies for hearing impairment.

Atrial natriuretic peptide (ANP) is a 28 amino acid peptide predominantly synthesized and secreted by the cardiac atria, and is the first member of the natriuretic peptide family, which also includes brain natriuretic peptide and C-type natriuretic peptide (de Bold et al., 1981). ANP interacts with two specific, high affinity receptors, NPR-A and NPR-C, on the plasma membrane of target cells to mediate its physiological effects (Levin et al., 1998; Potter et al., 2006, 2009). The natriuretic peptide receptor-A (NPR-A) is coupled to the particulate guanylyl cyclase (GC) which catalyzes the synthesis of the second messenger cyclic guanosine monophosphate (cGMP). cGMP modulates the activity of specific effector molecules including cGMP-regulated isoforms of phosphodiesterases, cyclic-nucleotide-gated ion channels, and cGMP-dependent protein kinases G, which in turn regulate diverse biological responses associated with blood vessel tone, neuronal excitability, transepithelial ion transportation, and the sensory transduction pathways underlying olfaction and vision (Potter et al., 2006, 2009). The natriuretic peptide receptor-C (NPR-C), which lacks the GC domain, contributes to the clearance of ANP and other natriuretic peptides from the circulation through receptor-mediated internalization and degradation. A number of studies have shown that NPR-C can also affect second messenger systems in a variety of tissues by inhibiting cyclic adenosine monophosphate (cAMP) production via a pertussis toxin-sensitive inhibitory guanine nucleotide regulatory protein (Gi), and by activating phospholipase-C without affecting cGMP levels (Anand-Srivastava and Trachte, 1993; Anand-Srivastava et al., 1990; Murthy et al., 2000; Rose and Giles, 2008).

In addition to the cardiovascular system, the tissue-specific distribution and function of ANP, NPR-A and NPR-C has been established in several tissues including kidney, adrenal, lung, adipose tissue and retina. Furthermore, ANP and its receptors have been found in the CNS, leading us to speculate that ANP may function as a neuromodulator or neuropeptide involved in neuronal and glial functions (Cao and Yang, 2008; Prado et al., 2010). Importantly, their presence in the secretory and sensory compartments of the rodent inner ear is well-documented, suggesting ANP may act as a local hormone regulating the fluid and electrolyte balance in the inner ear (Chen et al., 1994; Furuta et al., 1995; Koch et al., 1992; Krause et al., 1997; Lamprecht and Meyer zum Gottesberge, 1988; Meyer zum Gottesberge and Lamprecht, 1989; Meyer zum Gottesberge et al., 1991, 1995; Qiao et al., 2011; Seebacher et al., 1999; Suzuki et al., 1998; Yoon and Anniko, 1994; Yoon and Hellstrom, 1992). ANP receptors have been localized to the cochlear modiolus of the guinea pig (Lamprecht and Meyer zum Gottesberge, 1988) and rat spiral ganglion (Furuta et al., 1995). However, little is known regarding the localization and function of ANP and its receptors within the spiral ganglion.

We have previously provided direct evidence for the presence and synthesis of ANP as well as its receptors in cochlear SGNs (Sun et al., 2013). In our current study, we investigated the expression and localization of ANP and its receptors within the cochlear spiral ganglion of postnatal rat by immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis, with the aim of identifying their cellular localizations, expression levels and potential functions. We demonstrated that ANP and its receptors colocalized in both subtypes of SGNs and perineuronal SGCs within the spiral ganglion. Moreover, we revealed the differential expression patterns of ANP and its receptors at the mRNA and protein levels during postnatal development.

2. Materials and methods

2.1. Animals and tissue preparation

All cochleae used in this investigation were obtained from postnatal day 0, 7, 14, 21 and 28 (P0, P7, P14, P21 and P28) Sprague-Dawley rats (provided by the Laboratory Animal Center of the Fourth Military Medical University). The care and use of all animals in the study were carried out in accordance with the institutional guidelines of the Fourth Military Medical University. All efforts were made to minimize the number of animals used and to avoid their suffering. P0 rats were anesthetized on ice, while others were deeply anesthetized by intraperitoneally administrating an overdose of sodium pentobarbital (60 mg/kg). After anesthesia, the rats were decapitated and the skulls were opened midsagitally. With the aid of a dissecting microscope (SZX10; Olympus, Japan), the cochleae of rats at different ages were removed from the temporal bone, washed in ice-cold Hank's Balanced Salt Solution (HBSS; Invitrogen, USA) and collected for further use.

2.2. Immunofluorescent analysis

For immunohistochemistry, the cochleae from P0, P7 and P28 rats were fixed with 4% paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M, pH 7.2) by perfusion via the round and oval windows, and then incubated with the same fixative overnight at 4 °C. The cochleae were decalcified in a 5% EDTA solution for 2 days, followed by cryoprotection in 30% sucrose solution overnight at 4 °C. The samples were then embedded in Tissue-Tek OCT compound (Sakura Finetek, USA) at −20 °C, sectioned into 20 μm-thick mid-modiolar cross-sections using a cryostat microtome (CM1850; Leica, Germany) and mounted on poly-L-lysine-coated slides. After washing with phosphate buffered saline (PBS; 0.01 M, pH 7.4), all specimens were blocked with 5% normal donkey serum (NDS; Jackson ImmunoResearch, USA) and 0.1% Triton X-100 in PBS at 37 °C for 40 min, and incubated overnight at 4 °C with the following primary antibodies diluted in antibody solution (1% NDS and 0.1% Triton X-100 in PBS): polyclonal rabbit anti-ANP antibody (raised against amino acids 1–153 representing full length ANP of human origin; sc-20158, Santa Cruz, USA; 1:250), polyclonal goat anti-NPR-A antibody (raised against a peptide mapping within a cytoplasmic domain of NPR-A of human origin; sc-31632, Santa Cruz; 1:250), polyclonal goat anti-NPR-C antibody (raised against a peptide mapping within an internal region of NPR-C of human origin; sc-16871, Santa Cruz; 1:250), Alexa Fluor 488-conjugated polyclonal mouse anti-Tubulin β-III (TUJ1) antibody (detects the