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Research Paper

Ablation of the auditory cortex results in changes in the expression of neurotransmission-related mRNAs in the cochlea☆

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ABSTRACT

The auditory cortex (AC) dynamically regulates responses of the Organ of Corti to sound through descending connections to both the medial (MOC) and lateral (LOC) olivocochlear efferent systems. We have recently provided evidence that AC has a reinforcement role in the responses to sound of the auditory brainstem nuclei. In a molecular level, we have shown that descending inputs from AC are needed to regulate the expression of molecules involved in outer hair cell (OHC) electromotility control, such as prestin and the α10 nicotinic acetylcholine receptor (nAchR). In this report, we show that descending connections from AC to olivocochlear neurons are necessary to regulate the expression of molecules involved in cochlear afferent signaling. RT-qPCR was performed in rats at 1, 7 and 15 days after unilateral ablation of the AC, and analyzed the time course changes in gene transcripts involved in neurotransmission at the first auditory synapse. This included the glutamate metabolism enzyme glutamate decarboxylase 1 (glud1) and AMPA glutamate receptor subunits GluA2-4. In addition, gene transcripts involved in efferent regulation of type I spiral ganglion neuron (SGN) excitability mediated by LOC, such as the α7 nAchR, the D2 dopamine receptor, and the α1, and γ2 GABAA receptor subunits, were also investigated. Unilateral AC ablation induced up-regulation of GluA3 receptor subunit transcripts, whereas both GluA2 and GluA4 mRNA receptors were down-regulated already at 1 day after the ablation. Unilateral removal of the AC also resulted in up-regulation of the transcripts for α7 nAchR subunit, D2 dopamine receptor, and α1 GABAA receptor subunit at 1 day after the ablation. Fifteen days after the injury, AC ablations induced an up-regulation of glud1 transcripts.

1. Introduction

Previous studies have provided anatomical evidence for the presence of a descending efferent pathway originating in pyramidal neurons of layers V and VI of the auditory cortex (AC) reaching the cochlear receptor through connections with olivocochlear neurons (Mulders and Robertson, 2000b; Feliciano and Potashner, 1995). This pathway comprises downward projections with multiple feedback loops, including cortico-thalamic, cortico-collicular, cortico-(collicular)-olivocochlear and cortico-(collicular)-cochlear nucleus connections, that seem to work dynamically in combination (Bajo and Moore, 2005; Malmierca et al., 1996; Malmierca and Ryugo, 2011; Saldaña et al., 1996; Thompson and Schofield, 2000; Winer et al., 2001). The physiological effect of the cortical descending projections is to regulate the auditory signal processing in subcortical auditory nuclei (Liu et al., 2010; Luo et al., 2008; Suga et al., 2002; Yan et al., 2005; Lamas et al., 2013).

Recent studies have shown changes in the compound action potentials of the auditory nerve (Dragicevic et al., 2015; León et al., 2012), cochlear microphonics (Dragicevic et al., 2015; León et al., 2012; Xiao and Suga, 2002) and otoacoustic emissions (Jäger and Kössl, 2016; Khalfa et al., 2001; Perrot et al., 2006) after either AC activation or inhibition, thus demonstrating that the AC can

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modulate sensory transduction and neural conduction mechanisms in the initial auditory pathway levels.

We have previously reported an increase in auditory thresholds, and a decrease in both wave amplitudes and latencies of auditory brainstem responses after restricted ablations of the AC (Lamas et al., 2013). Due to the excitatory nature of the corticofugal projection (Feliciano and Potashner, 1995), the effect of AC removal on the activity of the cochlea and the auditory brainstem nuclei was interpreted as a result of the loss of descending excitatory inputs, ultimately affecting olivocochlear neurons. To test this, we previously analyzed the expression of molecular markers involved in the medial olivocochlear (MOC) - outer hair cells (OHC) neurotransmission, including prestin and the α10 nAchR subunit, after restricted AC ablations in rats (Lamas et al., 2015). Our results showed that AC ablations induce an increase in the transcripts of both prestin and α10 nAchR subunit, as well as change the oligomerization of the prestin protein, thus demonstrating a central role of the descending control in the regulation of the inner ear micromechanical machinery.

The loss of the descending control by ablation of the AC should also produce an imbalance in the efferent control of the type I spiral ganglion neurons (SGN), which may lead to changes in the expression of inner ear genes related to cochlear afferent signaling. In this report we test this using a similar experimental approach as in Lamas et al., 2015, to analyze the expression of molecular markers involved in the activity of the inner hair cells (IHC) - Type I SGN neurotransmission (Fig. 1). Thus, RT-qPCR was performed at 1, 7, and 15 days after unilateral ablations of AC, and the possible changes in transcripts involved in the IHC-Type I SGN neurotransmission, including the glutamate metabolism enzyme glutamate dehydrogenase (glud1) and the GluA2-4 AMPA receptor subunits (Kuriyama et al., 1994; Niedzielski and Wenthold, 1995) were analyzed. In addition, we also studied cochlear changes in transcripts involved in efferent regulation of type I SGN modulated by LOC, including α7 nAchR, D2 Dopamine receptor, and α1- and γ2-ionotropic GABAA receptor subunits (Morley et al., 1998; Maison et al., 2012; Drescher et al., 1993; Yamamoto et al., 2002; Ruel et al., 2000; Glowatzki and Fuchsa, 2002).

Our results showed that unilateral AC ablations induced up-regulation of GluA3 receptor subunit transcripts in the cochlea ipsilateral to the ablation, whereas both GluA2 and GluA4 mRNA receptors were down-regulated already at 1 day after the injury. Unilateral removal of the AC also resulted in up-regulation of the LOC post-synaptic transcripts α7 nAchR subunit, D2 dopamine receptor, and α1 GABAA receptor subunit at 1 day after the ablation. Fifteen days after the injury, AC ablations induced an up-regulation of glud1 transcripts. Similar changes for all the transcripts were observed in the cochlea contralateral to the AC ablation.

2. Methods

2.1. Animals

Twenty-eight male Wistar rats weighing between 250 and 300 g were used in this study. The animals were divided into sham controls, and three experimental groups. Animals from experimental groups had surgical ablation of the left AC and were randomly assigned to the different groups of survival times of 1, 7 and 15 days (n = 7 each one). Sham controls were animals undergoing the same surgery process than the experimental groups but without ablation of the AC. Sham controls were randomly assigned to the different groups of survival time. The comparison between them showed no differences in the level of the transcripts. Thus, we mixed them together and randomly selected 7 that we used as “controls” in the statistical analysis.

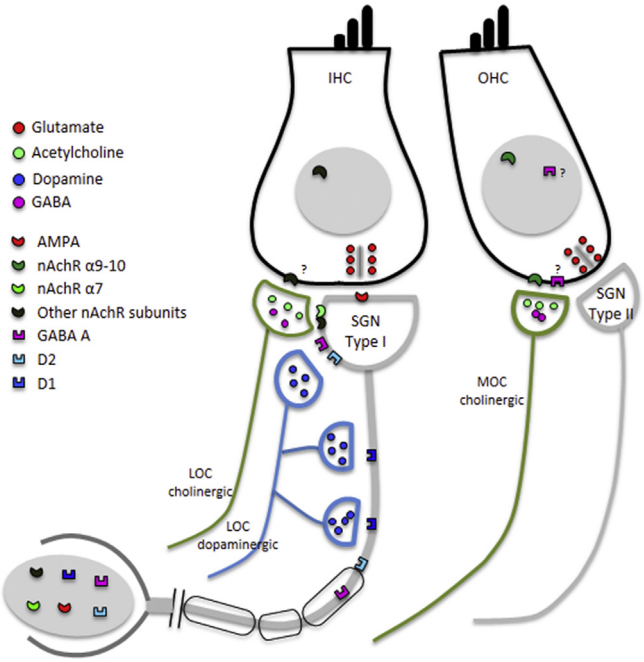

Fig. 1. Schematic of the neurochemical interactions among hair cells, spiral ganglion neurons, and olivocochlear efferent fibers in the mammalian cochlea. The connections and AMPA receptor location are known from ultrastructural (Liberman, 1980), as well as immunocytochemistry studies (Kuriyama et al., 1994; Niedzielski and Wenthold, 1995). The nature of the cholinergic receptors on Type I spiral ganglion neurons is inferred from the study of Morley et al., 1998, that combines RT-qPCR and in situ hybridization techniques. The nature of the cholinergic receptors on outer hair cells is known from electrophysiological (Elgoyhen et al., 2001) and knock-out studies (Maison et al., 2007; Vetter et al., 2007). Dopamine receptors location is known from the D1 and D2 receptor immunohistochemistry study of Maison et al. (2012). The gabaergic nature of the olivocochlear efferences and the location of the GABAA receptor subunits are known from immunohistochemistry studies (Maison et al., 2006, 2003; Vetter et al., 1991; Yamamoto et al., 2002). Dopamine and GABA presynaptic receptors located in LOC efferent terminals (Doleviczényi et al., 2005; Maison et al., 2012) are not schematized here. Receptors marked with a question mark indicate ambiguous localization.

This study was carried out in strict accordance with both Spanish regulations (Royal Decree 53/2013 - Law 32/2007) and European Union guidelines (Directive 2010/63/EU) on the care and use of animals in biomedical research.

2.2. Surgical procedures

AC ablations were performed under anesthesia using a mixture of ketamine chlorhydrate (30 mg/kg Imalgene 1000, Rhone Méreuse, Lyon, France) and xylazine chlorhydrate (5 mg/kg, Rompun, Bayer, Leverkusen, Germany), as previously described in Lamas et al. (2013). Briefly, animals were placed in a stereotaxic frame (#900, David Kopf Ins., Tujinga, CA, EEUU) and the left superficial area of the cranial surface was surgically exposed. A window including the primary and secondary AC areas was opened in the skull, following the stereotaxic coordinates of Paxinos and Watson, and the AC was removed by gentle aspiration. The animals were returned to their cages after the ablations, with careful monitoring of their post-surgery recovery. Once the corresponding post-surgery survival time was reached, the animals were anesthetized with 0.1 ml of sodium pentobarbital i.p., and decapitated in order to collect the brain and both cochleae. The brains were then immersed in 4% para-formaldehyde in PBS, while both ipsi- and contralateral cochleae from controls and surgically AC ablated animals were immediately frozen in liquid nitrogen.