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SCIENCE DIRECT
Hearing Research 216–217 (2006) 116–126
Hearing Research
www.elsevier.com/locate/heares

Research paper

Development of synapses and expression of a voltage-gated potassium channel in chick embryonic auditory nuclei

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Received 30 November 2005; received in revised form 12 January 2006; accepted 12 January 2006
Available online 10 March 2006

Abstract

The potassium channel protein, Kv3.1, is abundantly expressed in the chick auditory pathway. Its b-isoform is found in nucleus magnocellularis, which receives the cochlear input, both before and after the establishment of synaptic connections. It is also present in cell cultures in the absence of any peripheral input. However, the expression of this isoform in the embryo has been shown to increase with development. Here, we address the question of the correlation between maturation of synapses in the auditory pathway and the pattern of expression of the b-isoform in a series of embryos prepared for immunohistochemistry at Hamburger–Hamilton stages equivalent to E10, E12, E14, and E17. We show here that this subunit translocates from the perinuclear cytoplasm to the cell membrane domain in nucleus magnocellularis at the time that cochlear nerve endings emerge as endbulbs of Held (E17). In nucleus laminaris, by this time, while abundant Kv3.1b occurs in the perinuclear cytoplasm, a translocation to the cell membrane domain has not yet occurred, and the mature peri-synaptic localization is delayed to a later stage. This difference suggests a hierarchy in the developmental expression of Kv3.1. An unexpected finding is the expression of the a-isoform of Kv3.1 in astrocytes, especially those which surround the developing nuclei and their connecting fibers. We also report here for the first time the presence of Kv3.1b in the initial segments of axons at the times when they begin to form. Our observations suggest that the Kv3.1 channel protein is regulated through mechanisms linked to the development of synaptic activity.

Keywords: Kv3.1; Translocation; Nucleus magnocellularis; Nucleus laminaris; Astrocyte; Axon initial segment

1. Introduction

Kv3.1 is a member of the *Shaw* subfamily of voltage-gated potassium channels (Perny and Kaczmarek, 1991; Perny et al., 1992; Weiser et al., 1995; Perny and Kaczmarek, 1997; Li et al., 2001). A delayed rectifier, once activated, it rapidly repolarizes the cell membrane during an action potential so as to shorten its duration. This property ensures the ability for high frequency firing of the neuron (Reyes et al., 1994; Wang et al., 1998a; Gan and Kaczmarek, 1998; Rudy et al., 1999; Gurantz et al., 2000; Henne and Jeserich, 2004; Rudy and McBain, 2001; Macia et al., 2003). Kv3.1 is abundantly expressed in auditory neurons involved in preserving interaural-time differences (ITD) (Trussell, 1999; Parameshwaran-Lyer et al., 2001, 2003). In this pathway, the time interval between the arrival of a low frequency sound at each cochlea is encoded in the neural signal and this information is transmitted to higher centers of the brain for computing the location of the stimulus in relation to the head (Rose et al., 1967). It is thought that Kv3.1 is one of the crucial elements in maintaining the integrity of transmission in this ITD circuit (see Trussel and Parameshwaren-Lyer et al. above, also Gan et al., 1996; Carr et al., 2001; Ishikawa et al., 2003; von Hehn et al., 2004).

Abbreviations: NM, nucleus magnocellularis; NL, nucleus laminaris

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doi:10.1016/j.heares.2006.01.012

The nucleus magnocellularis (NM) is the avian homologue of the mammalian cochlear nucleus and the first station in the ITD pathway (Fig. 1). NM consists of a homogeneous population of bushy cells. The bushy cells receive the large endbulb synapses from the cochlear nerve fibers (Parks and Rubel, 1975; Rubel and Parks, 1975; Rubel et al., 1976; Jhaveri and Morest, 1982a,b; Cramer et al., 2000), which are responsible for preserving the precise timing of the discharges that are used for analysis of the ITD (Trussell, 1999; Brenowitz and Trussell, 2001; Carr et al., 2001; Rubel and Fritzsch, 2002). From the NM the neural signals go to the nucleus laminaris (NL), the avian homologue of the mammalian medial superior olive (Parks and Rubel, 1975; Jhaveri and Morest, 1982a). In the adult chicken, this nucleus consists of a single layer of neurons that have two sets of dendrites emanating respectively from the dorsal and ventral poles. The dorsal dendrites receive input from the ipsilateral NM, and the ventral dendrites receive from the contralateral NM (Fig. 1 inset). Thus, the binaural inputs impinge on NL and the first stage of computation takes place. This arrangement is thought to serve as the coincidence detection circuit in the ITD pathway (Jeffress, 1948; Joseph and Hyson, 1993; Carr and Boudreau, 1996; Carr et al., 2001; Rubel and Fritzsch, 2002).

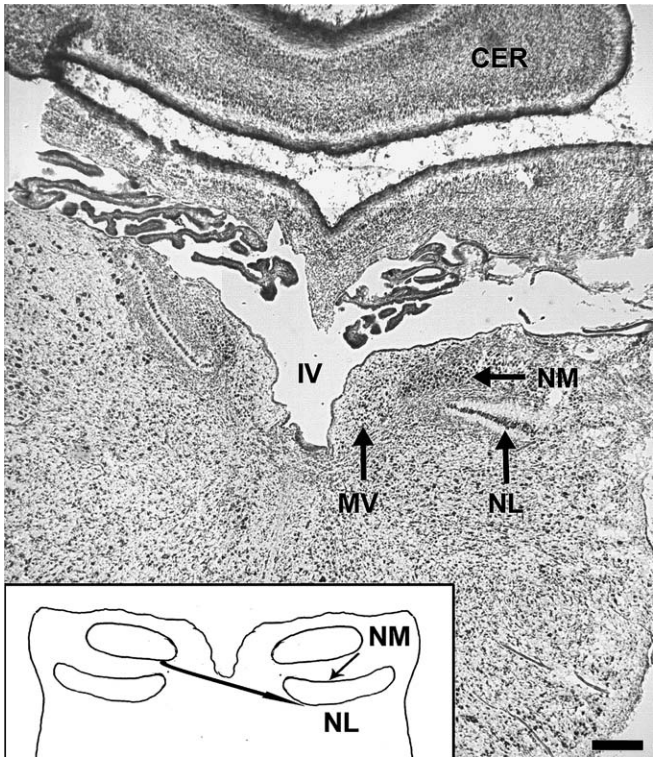

Fig. 1. Nucleus magnocellularis (NM) and nucleus laminaris (NL) in a Nissl-stained section cut in a plane perpendicular to the longitudinal axis of the hindbrain of a chick embryo at embryonic day 17 (E17). INSET: Diagram illustrating the projection from NM to NL. Other abbreviations: CER: cerebellum; IV, fourth ventricle; MV, medial vestibular nucleus. Scale: 200 μm. In this and in all of the figures, the dorsal direction is at the top of the field.

The anatomy and physiology of NM and NL have been extensively studied (see Morest, Rubel, Parks, and Carr above). Recently, the relationship between their physiology and potassium channels has received particular attention (Parameshwaran-Lyer et al., 2001, 2003). The early expression of Kv3.1 channel protein has been traced in the bushy cells of NM and principal cells of NL from E5 to E19, in the stages before and after the establishment of synaptic connections (Zhou et al., 2001). A normocytic culture system (Book et al., 1991) has been devised to study Kv3.1 expression in the absence of cochlear input (Feng and Morest, 2001) and with the patch-clamp technique in cells from the same tissue, as early as E2 (Hendriks et al., 1999a). These studies have shown that in the absence of synaptic input there is expression of Kv3.1 channel protein. There is also evidence of an up regulation of the conductance associated with Kv3.1 at the developmental stage when synapses form with the cochlear nerve (Hendriks et al., 1999a). In addition, findings by other authors suggest that depolarization can selectively increase the expression of Kv3.1 in auditory neurons (Liu and Kaczmarek, 1998b). In retinal ganglion cells, there is a coincidence between up regulation of Kv3.1 and the maturation of neurons (Henne and Jeserich, 2004). Taken together, all of these findings suggest that synaptic activity may play a role in regulating the expression of the Kv3.1 gene in the auditory pathway.

In the current study, we address the question of the developmental expression of Kv3.1 in relation to the maturation of synapses between cochlear nerve and NM, and between NM and NL. According to behavioral studies, this is the time when the chick embryo starts to show responses to acoustic stimuli (Jackson and Rubel, 1978). Physiological studies further showed that this is the period when the ability of transmission at the endbulb of Held exhibits marked improvement (Brenowitz and Trussell, 2001). We propose that, for a channel protein like Kv3.1, its presence and distribution in neurons would have to correlate with the ability of the neural circuit to transmit signals across synapses. As shown in the calyx of Held in rat, Kv3 potassium channels directly regulate the evoked release of transmitter (Ishikawa et al., 2003). Such a correlation may shed light on the regulation of the expression of this channel protein during development of NM and NL.

We show here that there is a correlation between the developmental expression of Kv3.1 and the maturation of synapses in NM and NL, although it is somewhat different in the two nuclei. We report the translocation of Kv3.1 from the neuronal cytoplasm to the cell membrane domain in NM during late embryonic development, consistent with the proposed function of this channel protein.

2. Materials and methods

Fertilized eggs from white leghorn chickens were obtained from The University of Connecticut poultry farm and incubated for various days until dissection. A series of