主論文の要旨

Auditory Input Shapes Tonotopic Differentiation of Kv1.1 Expression in Avian Cochlear Nucleus during Late Development

（発達期蝸牛神経核における聴覚入力依存的なカリウムチャネルの発現）

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Introduction

Tonotopic organization is a topographic representation of sound frequency in the auditory pathway, in which neurons are arranged orderly according to their characteristic frequency (CF). Recently, it was revealed that auditory neurons are differentiated morphologically and biophysically along the tonotopic axis. Moreover, these differentiations were suggested to depend on auditory experience. However, when and how auditory inputs contribute to the differentiations during development remain elusive.

Avian nucleus magnocellularis (NM) is a homologue of mammalian antero-ventral cochlear nucleus, and involved in relaying timing information of sound to higher auditory centers. NM is characterized by rich expressions of two types of voltage-gated-potassium channels, Kv1.1 and Kv3.1. Kv1.1 has a low-activation voltage and ensures phase-locked firing by suppressing aberrant spike generation, whereas Kv3.1 has a high-activation voltage and promotes high-frequency firing by accelerating falling phase of spikes, thereby playing an essential role in the precise temporal coding of the neurons. Among them, the expression of Kv1.1 was reported to differ tonotopically and to increase toward high-CF region. Thus, in this study, I characterized expressions of Kv1.1 and Kv3.1 at each CF region in NM of chickens during development, and examined their dependence on afferent inputs.

Materials and Methods

Chickens between embryonic day 12 (E12) and posthatch day 11 (P11), were used for whole-cell patch-clamp recording and immunohistochemistry.

Results

1) Major increase of potassium conductance occurred after hatch.

In current clamp recording, high- and low-CF NM neurons (Fig. 1B) generated one or a few spikes at the onset of depolarizing current after hearing onset (E12–14) (Fig. 1C, D). Threshold current and resting conductance increased during embryonic period, but the increase became much larger in posthatch period (Fig. 1E, F), particularly in high-CF neurons, indicating that major increase and differentiation of potassium conductance would progress after hatch in NM.

2) Tonotopic difference of Kv1.1 expression was largely created after hatch.

In voltage-clamp recording, an outward potassium current appeared during a depolarizing pulse, and an inward tail current followed after the pulse (Fig. 2A). Conductance-voltage curve of the tail current was fitted to a double Boltzmann equation, and components with half-activation voltage of -60 mV and -20 mV were defined as Kv1 and kv3 currents, respectively (Fig. 2C). Both Kv1 and Kv3 currents increased gradually during embryonic period to a slightly larger extent in high-CF neurons, while Kv1 current was far smaller

than Kv3 current (Fig. 2B, F). After hatch, however, Kv1 current showed a sharp increase in high-CF neurons, reaching 53% of total current (Fig. 2C, F), which largely created the tonotopic difference of the current in NM. On the other hand, Kv3 current decreased and did not show any tonotopic differences after hatch. Immunohistochemistry showed an increase of cytoplasmic signal for both Kv1.1 (Fig. 2D, G) and Kv3.1, while membranous Kv1.1 signal appeared and a ratio of membranous and cytosolic signals increased after hatch specifically in high-CF neurons (Fig. 2E, H). The results suggested that membranous translocation of Kv1.1 was accelerated around hatch in the high-CF neurons, which would underlie the tonotopic differentiation of Kv1.1 expression in mature animals.

3) Auditory inputs were required for the increase of Kv1.1 after hatch.

Auditory threshold is known to decrease around hatch. This raises a possibility that synaptic inputs to high-CF neurons increased around the period and mediated the increase of Kv1 current in the neurons. Thus, the auditory inputs were attenuated to three different levels after hatch (P0), by removing tympanic membrane (30 dB), fixing middle ear bone (columella, 50 dB), or removing cochlea (> 90 dB) (Fig. 3A, B). The attenuation of auditory inputs suppressed Kv1 current in a level-dependent manner particularly in high-CF neurons (Fig. 3G), and the amplitude was about half of control after cochlea removal (Fig. 3C, G). In addition both cytoplasmic and membranous signals of Kv1.1 decreased after the attenuation of auditory inputs (Fig. 3E, F, H, I), indicating that auditory inputs would accelerate Kv1.1 expression around hatch in the high-CF neurons. In contrast, elevation of auditory inputs by applying noise (100 dB) to animals for three days (P0–3) affected neither Kv1 current nor Kv1.1 signals, suggesting that Kv1.1 expression may reach a plateau level after hatch in NM (Fig. 3E, H).

During embryonic period, attenuation (bilateral otocysts removal) or elevation of auditory inputs had only minor effects on the expression of Kv1.1, indicating that contributions of auditory inputs would be small during the period.

Discussion

Expression of Kv1.1 developed slightly during embryonic period and this increase was rather independent of auditory inputs. On the other hand, Kv1.1 expression was accelerated greatly in high-CF neurons after hatch, and the increase depended strongly on auditory inputs, suggesting that auditory inputs in posthatch neurons should be critical in establishing the tonotopic differentiation of Kv1.1 in NM. Importantly, the input-dependence was strong in high-CF neurons even though they have a 20–30 dB higher threshold for sound than low-CF neurons. In addition, exposing embryos to noise could not induce the increase of Kv1 current. Therefore, one possible explanation for the increase of Kv1.1 expression in the posthatch high-CF neurons could be that cellular capacity to drive Kv1.1 expression in response to auditory inputs got strengthened around hatch and

augmented surface expression of the channel in the high-CF neurons. Surface expression of Kv1.1 is regulated via the ER retention domain at the external pore region, and proteins that bind to the domain, such as matrix metalloprotease 23, might be involved in this regulation.

Conclusion

Tonotopic differentiation of Kv1.1 is partially determined before hatch, but largely driven by afferent inputs after hatch. This differentiation would be attributable to the tonotopic-region-specific development of neuronal capacity to drive Kv1.1 expression via afferent inputs. The results would highlight the importance of neuronal character as well as auditory experience in the frequency tuning of auditory circuits.