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The Inner Hair Cell Synaptic Complex: Physiology, Pharmacology and New Therapeutic Strategies

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Key Words

Glutamate · Dopamine · Lateral olivocochlear system · Tonic inhibition · Synaptic transmission · Excitotoxicity · Cochlea

Abstract

Within the cochlea, the sensory inner hair cells (IHCs), which transduce mechanical displacement of the basilar membrane into neural activity, release glutamate to act on postsynaptic receptor channels located on dendrites of primary auditory neurons. In turn the activity of the postsynaptic auditory dendrites is modulated by a variety of lateral efferent neurotransmitters. This presentation reviews the most recent findings obtained at the IHC synaptic complex with an original technique, namely coupling auditory nerve single unit recordings with multibarrel intracochlear perfusions. Two types of results are emphasized: (1) in physiological conditions, the activity of auditory nerve fibers involves AMPA, but not kainate or NMDA receptors, and (2) this activity is tonically modulated by dopamine, one of the lateral efferent neurotransmitters. With the increasing knowledge of molecular mechanisms involved at the first synaptic complex in the cochlea, it is now possible to envisage local treatments for spiral ganglion neurons. These treatments, available experimentally, may be used in the near future: either to protect spiral ganglion neurons against excitotoxic injury (traumatic and/or ischemic sudden deafness), or to prevent excitotoxic-induced hyperexcitability (probably the starting point of most posttraumatic tinnitus), or to delay neuronal death (neural presbycusis).

Introduction

The inner hair cells (IHCs) are the mechanoelectrical transducers of the inner ear. They synapse with radial dendrites of the spiral ganglion neurons, and the auditory message is conveyed to the cochlear nucleus through the auditory nerve. In turn, the lateral efferent system, originating from the lateral superior olive, synapses on auditory nerve dendrites underneath IHCs. The so-called IHC synaptic complex is formed of an afferent synapse (IHC/auditory dendrite) and an efferent synapse (lateral efferent ending/auditory dendrite).

Glutamate (Glu) is accepted to be the main neurotransmitter at the IHC-auditory nerve synapse [Puel, 1995]. Based on chemical neuroanatomy results [Eybalin, 1993], current knowledge on efferent neurotransmitters can be summarized as follows. The lateral efferents may use several neurotransmitters or neuromodulators [acetylcholine, GABA, dopamine (DA), enkephalins, dynorphins and calcitonin gene-related peptide]. To date, very little is known about the roles of these lateral efferent neurotransmitters. This report summarizes recent neuropharmacological data on the IHC afferent/efferent synaptic

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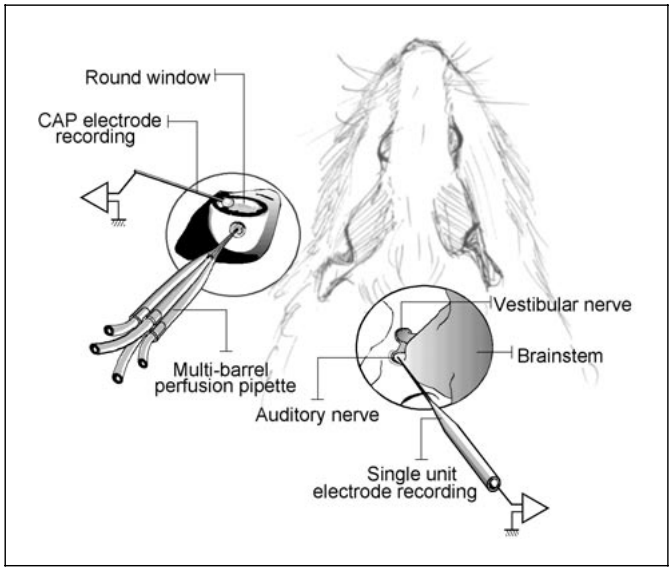

Fig. 1. Schematic representation of the design of the experiment: the perilymphatic perfusion technique. The cochlea was exposed using a dorsal approach (left side of the figure). A 0.5-mm hole was gently drilled into the scala tympani of the basal turn of the cochlea to receive a multibarrel perfusion pipette (ASI Instruments) and a CAP recording electrode placed on the round window of the cochlea. The test solutions were allowed to flow exposed using a posterior fossa approach (right side of the figure). Unit activity was tracked by advancing the microelectrode through the cochlear nerve with a motorized micromanipulator (Micro-control, module 80) during exposure to 80 dB SPL white noise generated by a Brüel & Kjær (type 1405; bandwidth 100 kHz). Once a single unit was isolated, spontaneous activity was averaged over 10 s.

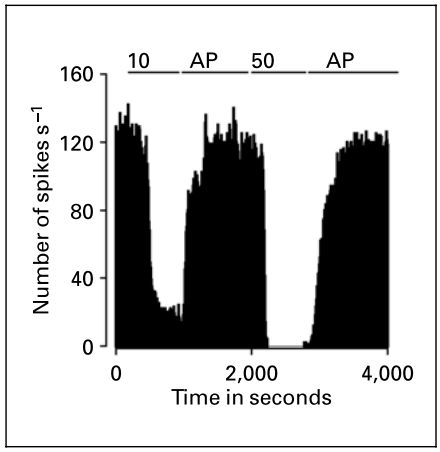

Fig. 2. Effect of GYKI 53784 on the spontaneous single unit activity of an auditory nerve fiber. The spontaneous activity of an auditory nerve fiber during a 10-min perfusion of control artificial perilymph (AP) and AP containing 10 and 50 μ*M* GYKI 53784 is shown. Application of 10 μ*M* of GYKI 53784 provoked a drastic reduction in the spontaneous activity of the auditory nerve fiber. This effect was completely reversed when the cochlea was rinsed with control AP. When the sequence was repeated with 50 μ*M* GYKI 53784, spontaneous activity was completely abolished and full recovery was obtained after a 10-min perfusion with control AP.

complex, including the type of Glu receptors involved and their modulation by DA. Neuropharmacological data were obtained by combining the recording of a single unit of the auditory nerve with the intracochlear application of drugs via a multibarrel pipette (fig. 1). Subsequently, histological examination (electron microscopy) of synapses was performed to check for excitotoxicity.

Pharmacology of the Glutamatergic Synapse

Analysis of ionotropic Glu receptors with gene expression, immunocytochemistry and in situ hybridization indicates that primary auditory nerve cells express NMDA (NR1 and NR2A–D), AMPA (GluR2–4) and kainate (GluR5–7) receptor subunits, as well as the high affinity kainate binding proteins (KA1 and KA2) [Ryan et al., 1991; Safieddine and Eybalin, 1992; Niedzielski and Wenthold, 1995; Usami et al., 1995; Matsubara et al., 1996]. This suggests that NMDA, AMPA and kainate receptors might coexist on primary auditory nerve cells. However, the role of each of these receptors at the IHC-to-auditory nerve cell synapse is controversial and unsettled. For instance, although iontophoretic application of NMDA appears to induce excitation of the primary auditory nerve fibers [Felix and Ehrenberger, 1990], no effect of NMDA has been observed on isolated primary auditory nerve soma [Nakagawa et al., 1991; Ruel et al., 1999] or in intact preparations [Ruel et al., 2000]. Another reason for this controversy is that, in addition to activating kainate receptors, kainate can also act on the AMPA receptors [Boulter et al., 1990; Keinänen et al., 1990; Patneau and Mayer, 1991]. Isolated primary auditory nerve soma have been shown to respond to Glu and AMPA by a fast onset inward current that was rapidly desensitized, while kainate induced only a nondesensitizing, steady-state current [Nakagawa et al., 1991; Ruel et al., 1999]. Recently, GYKI 53784 (LY303070) has been demonstrated as being one of the most selective antagonists for AMPA recep-

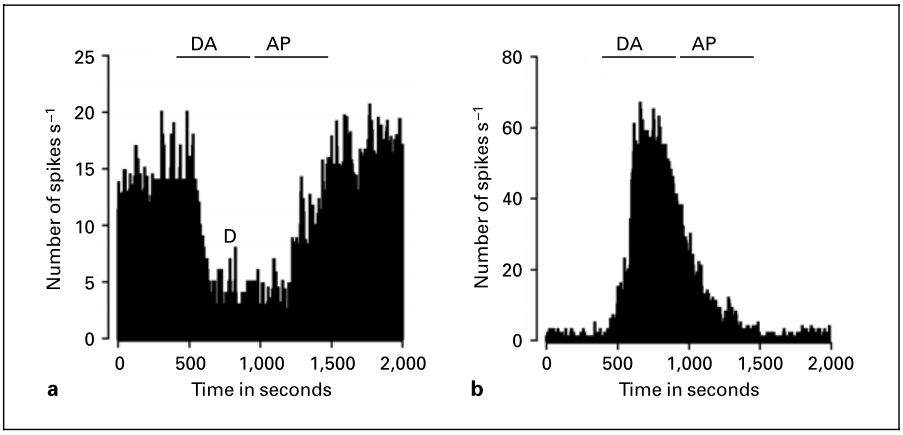

Fig. 3. Effect of DA and D2-like antagonist eticlopride (ETI) on single unit activity. **a** 10-min perfusion with artificial perilymph (AP) containing 1 m*M* DA reversibly decreases the spontaneous rate. **b** The spontaneous activity was recorded during a 10-min perfusion of 50 μ*M* ETI. Note the large increase in the discharge rate from 5 to 60 spikes/s, reversed after rinsing the cochlea with AP.

tors [Bleakman et al., 1996a]. Taking advantage of this new pharmacological tool, the role of AMPA receptors in the cochlear fast synaptic transmission was addressed. GYKI 53784 (LY303070) was compared with additional AMPA/kainate antagonists, GYKI 52466 and DNQX, and with the NMDA antagonist D-AP5 [Ruel et al., 1999, 2000].

Although the NMDA antagonist D-AP5 had no effect on cochlear potentials, GYKI 53784 had the same potency as DNQX in reducing the compound action potential (CAP). Its effect on the spontaneous activity of the single auditory nerve fibers was studied (fig. 2). Perfusion of 10 μ*M* GYKI 53784 drastically reduced the spontaneous discharge rate of the auditory nerve fibers. In cases where the fiber was maintained for a sufficient amount of time, the drug was washed out and the same paradigm was repeated with 50 μ*M* of GYKI 53784. The activity of the fiber was completely abolished by 50 μ*M* of GYKI 53784, suggesting that fast excitatory neurotransmission between the IHCs and the primary auditory nerve fibers is predominantly mediated by AMPA-preferring receptors, and not by kainate or NMDA receptors.

Modulation of Glutamatergic Synapses by DA

Most of the data dealing with lateral olivocochlear (LOC) efferents come from studies after lesions of the olivocochlear bundle. These results should be interpreted with caution due to technical difficulties in the surgery, especially when trying to selectively severe the lateral and the medial efferent systems. Moreover, the olivocochlear fibers branch extensively upon entering the organ of Corti, and even a few remaining fibers may be sufficient to maintain normal function. To date, only two studies report changes in auditory nerve responses following complete cochlear de-efferentation in adult animals [Liberman, 1990; Zheng et al., 1999]. In these investigations, sectioning the entire olivocochlear bundle resulted in a slight, but significant change in threshold (within 10–15 dB), and in a drastic decrease in the spontaneous discharge (all fibers had spontaneous rates below the normal mean).

Due to these technical limitations, we decided to study the LOC system by using an in vivo pharmacological approach in adult guinea pig. The LOC innervation may use several neurotransmitters or neuromodulators (acetylcholine, GABA, DA, enkephalins, dynorphins and calcitonin gene-related peptide). In contrast to acetylcholine and GABA that are also used in the medial olivocochlear system, DA is present only in the LOC system [see Eybalin, 1993, and Puel, 1995, for review]. Consequently, the present study focussed on DA to examine the functional role of an LOC neurotransmitter on auditory nerve activity [Ruel et al., personal commun.].

When perfused into the cochlea, 1 m*M* DA induced a reduction in spontaneous firing (fig. 3a). This effect was completely reversed by washing DA out of the cochlea with control artificial perilymph. To assess the role of endogenous DA, we used specific DA antagonists, such as eticlopride [see Missale et al., 1998, for review]. The effect differed depending on the two classes of fibers [Liberman, 1978]: low spontaneous rate (<20 spikes/s) and high spontaneous rate (>20 spikes/s). In low spontaneous rate fibers, DA blockade with eticlopride resulted in an increase of the spontaneous firing with little or no postexcitatory suppression (fig. 3b). In high spontaneous rate fibers, eticlopride induced a brief increase in firing rate,

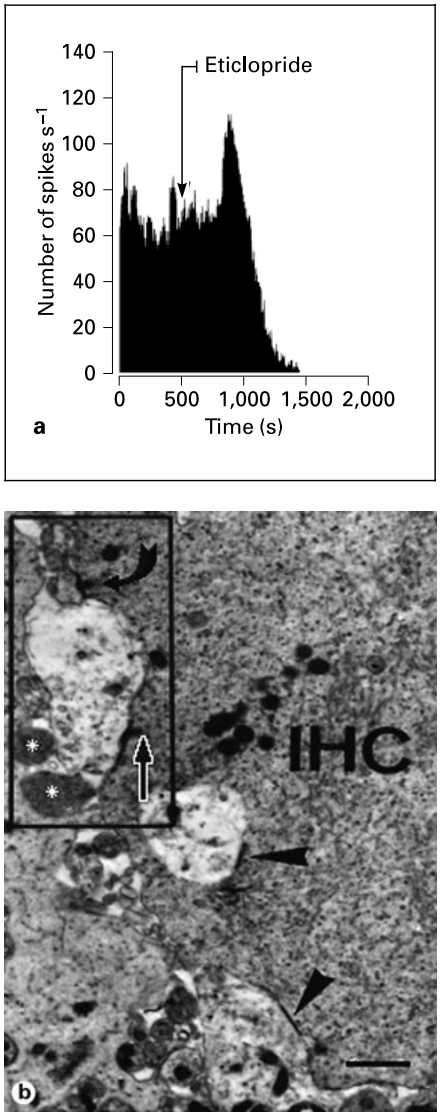

Fig. 4. Effect of D2 antagonist eticlopride on high spontaneous firing rate fibers and on the ultrastructure of the organ of Corti. **a** 10-min perfusion with artificial perilymph containing 50 μ*M* eticlopride induces a fast transient increase in spontaneous activity followed by a marked reduction in firing rate. **b** Electron micrograph of the synaptic pole of an IHC in the upper part of the cochlear basal turn of the same guinea pig (after a 10-min perfusion of 50 μ*M* eticlopride). Two afferent endings are seen connecting the IHC base. No changes are seen on the presynaptic side (arrows point to normal synaptic bodies and arrowheads to presynaptic densities). Due to eticlopride and removal of DA inhibition, most of the postsynaptic endings (afferent dendrites) are swollen. The frame on the upper left points to two different auditory dendrites: a small unaffected bouton (top, curved arrow); a swollen dendrite (bottom, straight arrow) connected by two efferents (asterisks). Bar: 0.5 μm.

immediately followed by a reduction to values below predrug rates (fig. 4).

To investigate the mechanism underlying this postexcitatory inhibition, cochleas perfused with eticlopride were fixed and processed for transmission electron microscopy. While no abnormality could be detected in the cochlea perfused with artificial perilymph (fig. 4a), a clear swelling of some of the afferent dendrites connected to the IHCs was observed in the cochlea perfused with DA antagonist eticlopride (fig. 4b). The swollen radial dendrite terminals exhibited disarray of their cytoskeleton (fig. 4c). IHCs showed normal presynaptic structure facing these swollen radial dendrites. Similarly, normal looking vesiculated efferents made synaptic contact with the swollen radial dendrites. This type of damage has also been reported after a local application of Glu, AMPA and kainate, but not NMDA, and has been referred to excitotoxic injury [Ruel et al., 2000]. This suggests that the marked reduction in firing rate observed on the higher preperfusion rate fibers may reflect early signs of excitotoxicity that occurred during the application of eticlopride. Consistent with this assumption, the AMPA antagonist GYKI 53784 blocked eticlopride-induced swelling of afferent dendrites (not shown).

Synaptic Plasticity and Tinnitus

Since we reported a structural and functional recovery after local application of AMPA [Puel et al., 1995], several studies have confirmed this regenerative capacity of auditory neurites using different excitotoxic protocols, such as a local application of kainate [Zheng et al., 1997] or a noise trauma [Puel et al., 1998]. To summarize, after a massive destruction of all dendrites of the primary auditory neurons connected with the IHCs, there is a regrowth and a neoformation of synapses accounting for functional recovery. Here we would like to emphasize two points during this process of synaptic repair, namely the respective roles of NMDA receptors and of the lateral efferent neurotransmitters, which may account for pathological activities in the auditory neurons.

Role of NMDA Receptors

In situ hybridization experiments performed in the cochleas during the reinnervation process have revealed an increased expression of mRNA coding for NR1 subunit NMDA receptors in spiral ganglion neurons [Puel et al., 1995] (fig. 5). The results suggest that NR1 receptors are involved in the regeneration of neurites and in the for-

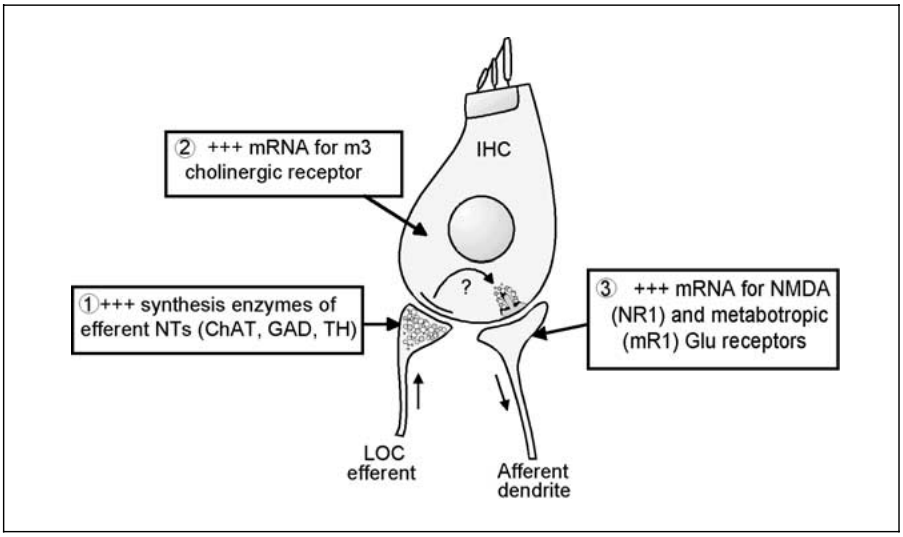

Fig. 5. Plasticity at the IHC synaptic complex after excitotoxicity. After an excitotoxic injury, during and just after the process of neosynaptogenesis between the auditory (afferent) dendrite and the IHC, the lateral (LOC) efferent endings directly contact the base of the IHC, where postsynaptic cisternae are visible [Pujol et al., 1996]. Meanwhile, several neurochemical changes affect all components [Eybalin et al., 1995]: (1) Within the LOC efferent neurons, there is an increase in the immunolabeling of the synthetic enzymes for acetylcholine (ChAT), GABA (GAD), and DA (TH). (2) Within the IHC, in situ hybridization shows upregulation of m3 cholinergic receptor mRNA. (3) Within spiral ganglion neurons, in situ hybridization indicates upregulation of mRNAs for NMDA (NR1) and metabotropic (mGluR1) glutamate receptors. It is possible that there is direct stimulation of the IHC by the transient efferent synapses, bypassing sound-induced transduction (curved arrow with question mark). Together, these may account for hyperexcitability and abnormal firing in the auditory nerve fibers, possibly perceived as a tinnitus. If so, appropriate neurochemical targets are already available.

mation of new synapses. Indeed, the blockage of NMDA receptors delays the regrowth of neurites, delays the formation of new synapses, and delays the restoration of hearing [d'Aldin et al., 1997]. Thus glutamate, beside its damaging excitotoxic effect, may play a neurotrophic role during synaptogenesis in the posttraumatic cochlea, via an activation of NMDA receptors. Moreover, such an overexpression of NMDA receptor after excitotoxic injury may well account for an epileptic-like firing of the auditory nerve and be the starting point of a peripheral tinnitus.

Role of Lateral Efferent Neurotransmitters

During the reinnervation process we described the presence of an abnormally high number of lateral efferent varicosities bearing an electron-dense appearance. Several of them were in close contact with the IHCs in which typical postsynaptic cisternae could be observed [Puel et al., 1995; Pujol et al., 1996], mimicking what has been described during developmental synaptogenesis [Pujol et al., 1997]. In adult cochleae, direct contacts between efferent varicosities and the base of IHCs are sparse and they generally cannot be described as 'synapses' in serial sections [Liberman, 1980].

Interestingly, this structural result correlates with two sets of neurochemical observations (fig. 5): (1) a significant upregulation of synthetic enzymes for the efferent neurotransmitters (tyrosine hydroxylase, choline acetyl transferase, glutamic acid decarboxylase) [Eybalin et al., 1995], and (2) an upregulation of mRNA for m3 cholinergic receptors in the IHC [Eybalin and Saffiedine, personal commun.]. A working hypothesis is that direct activation of IHCs by lateral efferents, enhancing Glu release, may be involved in the growth and the regrowth of neurites. In addition this Glu release occurs whilst postsynaptic neurites overexpress NMDA receptors [Puel et al., 1995; d'Aldin et al., 1997]. This abnormal circuitry at the base of IHCs may also be involved in the origin of some forms of peripheral tinnitus.

Conclusions

Here we confirm that AMPA receptors, and not kainate or NMDA receptors, mediate fast excitatory transmission at the IHC-auditory nerve synapse. Moreover, the activity of AMPA receptors seems to be tonically

modulated by the dopaminergic lateral efferent system, which acts as a permanent gain control at the initialization site of the auditory action potential. This gain control is responsible for the maintenance of fundamental characteristics of auditory nerve responses. Dysfunction of this system leads to the development of early signs of glutamate-induced excitotoxicity. These results, adding to the knowledge of the molecular mechanisms of the synaptic plasticity (fig. 5), indicate that, in the near future we could well expect some new therapeutic strategy applicable in humans. For instance, after ischemic and/or noise-induced hearing loss (sudden deafness), it should be possible to help the protection and/or the repair of synapses, the regrowth of nerve fibers, and functional recovery. Similarly, other treatments for spiral ganglion neurons are conceivable, either to stop an excitotoxic-induced hyperexcitability (probably the starting point of most posttraumatic tinnitus), or to prevent neuronal death (neural presbycusis). Based upon experimental results reported here and in related papers, this new therapeutic strategy should be developed using a local application of drugs, as via a trans-tympanic catheter, allowing precise targeting of the molecular mechanism involved, and the use of concentrations low enough to avoid side effects.

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