Excitotoxicity, Synaptic Repair, and Functional Recovery in the Mammalian Cochlea: A Review of Recent Findings

RÉMY PUJOL[a] AND JEAN-LUC PUEL

INSERM and University Montpellier 1, Montpellier, France

ABSTRACT: Besides its fast excitatory properties, glutamate is known to have neurotoxic properties when released in large amounts or when incompletely recycled. This so-called excitotoxicity is involved in a number of acute and/or degenerative forms of neuropathology such as epilepsy, Alzheimer's, Parkinson's, stroke, and retinal ischemia. In the cochlea, excitotoxicity may occur in two pathological conditions: anoxia and noise trauma. It is characterized by a two-step mechanism: (1) An acute swelling, which primarily depends on the AMPA/kainate type of receptors, together with a disruption of the postsynaptic structures (type I afferent dendrites) resulting in a loss of function. Within the next 5 days, synaptic repair may be observed with a full or a partial (acoustic trauma) recovery of cochlear potentials. (2) The second phase of excitotoxicity, which may develop after strong and/or repetitive injury, consists of a cascade of metabolic events triggered by the entry of Ca^{2+}, which leads to neuronal death in the spiral ganglion. Ongoing experiments in animals, tracking the molecular basis of both these processes, presages the development of new pharmacological strategies to help neurites to regrow and reconnect properly to the IHCs, and to prevent or delay neuronal death in the spiral ganglion. Human applications should follow, and a local (transtympanic) strategy against cochlear excitotoxicity may, in the near future, prove to be helpful in ischemic- or noise-induced sudden deafness, as well as in the related tinnitus.

INTRODUCTION

Synapses between the inner hair cells (IHCs) and the auditory dendrites are glutamatergic (see references 1 and 2 for recent reviews), which means that they are run by the so-called glutamate receptors at both pre- and postsynaptic sides. Besides its fast excitatory properties, glutamate is well known to have neurotoxic properties when excessively released or incompletely recycled: this is called excitotoxicity (see, among others, references 3 and 4). Actually, excitotoxicity has been involved, either directly or in addition to other factors, in a number of acute and/or degenerative forms of neuropathology such as epilepsy, Alzheimer's, Parkinson's, stroke, retinal ischemia, and viral encephalopathy (see reference 5 for a review). In the cochlea, excitotoxicity may occur in two pathological conditions: anoxia[6,7] and noise trauma.[8,9]

[a]Address for correspondence: INSERM Unité 254, 71 rue de Navacelles 34090, Montpellier, France. Phone: +33 467 417 702; fax: +33 467 417 720; e-mail: rpujol@montp.inserm.fr

As in other parts of the nervous system, it is characterized by a two-step mechanism:[3] (1) due to massive ion and water entry, an acute swelling and disruption of the postsynaptic structures (i.e., type I afferent dendrites connected to the IHCs) results in a loss of function; and (2) triggered by Ca^{2+} entry, a cascade of metabolic events could follow, including free-radical production, protease, and endonuclease activation, eventually leading to neuronal (i.e., type I spiral ganglion neuron) death. We have further investigated the acute cochlear excitotoxicity by either direct application of glutamate agonists,[7] after ischemia,[6,7] or after noise exposure.[8,9] In all conditions, the type I auditory dendrites reacted in a dose-dependent manner, with an increased swelling, then a membrane rupture, of the postsynaptic compartment. This acute swelling depends primarily on the AMPA/kainate type of receptor, as DNQX (a selective AMPA antagonist) or kynurenate (a large spectrum antagonist), but not D-AP5 (an NMDA antagonist), block the excitotoxic processes. Within the next 5 days, however, a recovery of both the normal pattern of IHC innervation was observed,[10] based upon regrowth of auditory dendrites and neoformation of synapses with the IHCs. This process of synaptic repair was accompanied by either a full recovery of cochlear potentials (AMPA application), or a partial (around 50%) recovery (acoustic trauma). In this later case, mechanical damage, mainly to the outer hair cells (OHCs) and/or their stereocilia, is responsible for a second and slower phase of recovery lasting 10 to 15 days, which could be incomplete (PTS) when some OHCs have been killed. Because the synaptic repair mechanism after excitotoxicity mimics the normal development, its molecular bases are similar, and therefore some pharmacology can be developed to help neurites to regrow and reconnect properly with the IHCs. Moreover, the second phase of excitotoxicity, which has a chance to develop after strong and/or repetitive excitotoxic attacks, might well be prevented or delayed by, for example, procedures similar to those used to protect CNS neurons after a stroke. All the conditions are there right now to begin developing a local (transtympanic) pharmacological strategy against cochlear excitotoxicity in case of ischemic- or noise-induced sudden deafness, as well as for related tinnitus.[11]

MECHANISM OF COCHLEAR EXCITOTOXICITY

In the adult cochlea, a local application of glutamate agonists, kainate[12] or AMPA,[7] causes an immediate and massive swelling of radial auditory dendrites from type I spiral ganglion neurons, which are exclusively connected to the IHCs. The AMPA-induced swelling is dose-dependent and can be prevented by a prior perfusion of the specific antagonist 6-7-dinitroquinoxaline-2,3-dione (DNQX[7]). On the other hand, the same radial afferent endings connected to IHCs have been described as being very sensitive to ischemia.[6,13] Accordingly, these fibers appeared swollen in all cases in which ischemic or anoxic conditions preceded cochlear fixation. The similarity of the acute damage on the radial dendrites caused by either glutamate agonists or ischemia is so striking that we decided to investigate the hypothesis that a similar mechanism of amino-acid excitotoxicity is involved in both conditions. This seems to be the case, since a prior application of DNQX has the same protective effect on the AMPA-induced and ischemia-induced swelling.[6,7] Finally, it is now well established

that besides mechanical alteration, mainly at the OHC level, noise trauma is involved in postsynaptic damage at the base of IHCs.[8,9,14,15] This consists of a disruption of the primary auditory dendrites below the IHCs, leading to synaptic uncoupling and a loss of function. Again, the similarities of both the damage and the protective effect of glutamate antagonists suggest a common mechanism. The high degree of protection observed with the broad spectrum glutamate antagonist kynurenate (about 50%) indicates that dendritic damage is an important component in noise-induced hearing loss.[8,9]

PROTECTIVE ROLE OF THE LATERAL EFFERENT SYSTEM

The lateral efferent innervation may well represent a built-in mechanism of protection against excitotoxicity of the radial afferent fibers connected to the IHCs. This efferent innervation uses classic neurotransmitters (acetylcholine, GABA, and dopamine), as well as neuropeptides (enkephalins, dynorphins, and calcitonin gene-related peptide), and most of these substances seem to colocalize within the same neurons and/or terminal.[1] Although the functional role of these neurotransmitters/neuromodulators needs to be clarified, recent data favor the hypothesis that at least dopamine is involved in protecting the cochlea against noise,[16,17] likely by reducing the noise-induced excitotoxicity at IHC synapses.[18] To summarize these data, an intracochlear perfusion of 1 mM of piribedil (a D2/D3 agonist) prevented the damaging effect on auditory dendrites of either 10-min ischemia, or a noise trauma,[18] in the same way as DNQX[6] or kynurenate,[8] respectively. It is thus possible that the dopaminergic lateral efferent could, to a certain extent, protect the primary auditory neurons via D2/D3 receptors. Although D2/D3 receptors are generally associated with the inhibition of adenylate cyclase activity, the exact mechanism by which piribedil maintains the osmotic balance in primary auditory dendrites and, thus, protects them against excitotoxicity still remains unknown.

SYNAPTIC REPAIR AND FUNCTIONAL RECOVERY

Since we reported a functional and structural recovery after local application of AMPA,[10] several studies have confirmed this regenerative capacity of auditory neurons using different excitotoxic protocols, such as a local application of kainate[19] or a noise trauma.[9]

Our paper[10] can be summarized as follows. Guinea-pigs were implanted with an osmotic micropump containing artificial perilymph. They received an acute perfusion of AMPA, and showed no measurable CAP immediately after the perfusion: a lack of electrophysiological response clearly correlated with a massive destruction of all dendrites of the primary auditory neurons connected with the IHCs. The physiological follow-up of these animals showed a 30% recovery of the CAP one day after the AMPA perfusion. At this stage, newly formed dendrites were seen in contact with the IHC base that had already formed some rare synapses. Interestingly, the presence of some branching of regenerating dendrites and vesiculated efferents in direct contact with the IHCs is clearly reminiscent of early developing cochlear synapses (see

reference 20 for review). At 5 days postexposure, the pattern of innervation of the IHC looked normal, thus accounting for the complete recovery of the CAP. Typical synaptic specializations were observed where auditory nerve endings contacted IHCs. The regenerated dendrites were also normally contacted by efferent endings. *In situ* hybridization experiments performed in these cochleas during the reinnervation process have revealed an increased expression of mRNA coding for the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors in spiral ganglion neurons. These results suggest that NR1 receptors are implicated in the neuronal regenerative process, and the formation of synapses.

EFFECTS OF D-AP5 ON THE FUNCTIONAL RECOVERY AFTER AMPA

We have tried to understand some of the molecular mechanisms underlying the synaptic repair. Because our *in situ* hybridization data suggested an involvement of NMDA receptors,[10] we have applied the NMDA antagonist D-AP5 with an osmotic micropump during the functional recovery. This resulted in a delayed restoration of cochlear function until the pump was stopped. Indeed, instead of recovering normal function within the 5 days after AMPA exposure, the guinea pigs that received D-AP5 for 5 days showed only about 20% CAP recovery by the fifth day, with potentials not reaching normal values until between days 10 to 15.[21] Morphological investigation in the D-AP5-treated cochleas 5 days after AMPA revealed features that have already been described in earlier stages of synaptic repair (i.e., one day after AMPA exposure). This result demonstrates that the blockage of the NMDA receptors during functional recovery delays the regrowth of neurites, the neoformation of synapses, and the restoration of hearing. It suggests that glutamate, besides its damaging excitotoxic effect, may play a neurotrophic role during the cochlear posttraumatic synaptogenesis via an activation of NMDA receptors. Such a neurotrophic role for NMDA receptors during synaptogenesis is already well documented (see reference 22 for review). Other experiments to investigate the role of different subunits of glutamate receptors are in progress. Similarly, we are also investigating the possible involvement of an NMDA receptor activation in microtubule-associated protein (MAP2) expression. Preliminary results indicate that "early" MAP2c is involved in dendritic growth, while the "late" MAP2a,b are linked to the stabilization of synapses.

CONCLUSIONS

Understanding the mechanism of IHC reconnection by the primary auditory dendrites, after they have been damaged by excitotoxicity, is a very important issue for the development of future therapies. Indeed, dendritic damage linked to glutamate excitotoxicity could be involved in various cochlear pathologies such as ischemia- or noise-induced sudden deafness, and neural presbycusis. Auditory dendrites, for instance, are disrupted after an acoustic trauma, overstimulating the glutamatergic IHCs. Consequently, part of the reversibility of the hearing loss observed after noise exposure could be explained by a reconnection of the IHCs by the auditory dendrites.[9] Similarly, some forms of sudden deafness could be due to an ischemia-in-

duced excitotoxicity, reversible cases being probably accounted for by regenerative mechanisms within the primary auditory neurons. Finally, a cumulative effect of repeated excitotoxic injuries may well result in triggering the second phase of the excitotoxic process, that is, spiral ganglion neuron death. This is a possible explanation for the reported cases of neural presbycusis with a normal counting of IHCs, but a 50% reduction in ganglion neurons.[23] The increasing knowledge about molecular mechanisms of nerve regeneration and neuronal protection is promising for future clinical applications. However, future progress in the field will certainly require that a method, likely transtympanic, be developed for the local application of drugs in human subjects.

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