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PROTECTIVE EFFECT OF RASAGILINE IN AMINOGLYCOSIDE OTOTOXICITY

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Abstract—Sensorineural hearing losses (SNHLs; e.g., ototoxicant- and noise-induced hearing loss or presbycusis) are among the most frequent sensory deficits, but they lack effective drug therapies. The majority of recent therapeutic approaches focused on the trials of antioxidants and reactive oxygen species (ROS) scavengers in SNHLs. The rationale for these studies was the prominent role of disturbed redox homeostasis and the consequent ROS elevation. Although the antioxidant therapies in several animal studies seemed to be promising, clinical trials have failed to fulfill expectations. We investigated the potential of rasagiline, an FDA-approved monomanine oxidase type B inhibitor (MAO-B) inhibitor type anti-parkinsonian drug, as an otoprotectant. We showed a dose-dependent alleviation of the kanamycin-induced threshold shifts measured by auditory brainstem response (ABR) in an ototoxicant aminoglycoside antibiotic-based hearing loss model in mice. This effect proved to be statistically significant at a 6-mg/kg (s.c.) dose. The most prominent effect appeared at 16 kHz, which is the hearing sensitivity optimum for mice. The neuroprotective, antiapoptotic and antioxidant effects of rasagiline in animal models, all targeting a specific mechanism of aminoglycoside injury, may explain this otoprotection. The dopaminergic neurotransmission enhancer effect of rasagiline might also contribute to the protection. Dopamine (DA), released from lateral olivocochlear (LOC) fibers, was shown to exert a protective action against excitotoxicity, a pathological factor in the aminoglycoside-induced SNHL. We have shown that rasagiline enhanced the electric stimulation-evoked release of DA from an acute mouse cochlea preparation in a dose-dependent manner. Using inhibitors of voltage-gated Na^+-, Ca^{2+} channels and DA transporters, we revealed that rasagiline potentiated the action potential-evoked release of DA by inhibiting the reuptake. The complex, multifactorial pathomechanism of SNHLs most likely requires drugs acting on multiple targets for effective therapy. Rasagiline, with its multi-target action and favorable adverse effects profile, might be a good candidate for a clinical trial testing the otoprotective indication.

Key words: sensorineural hearing loss, kanamycin, auditory brainstem response, lateral olivocochlear efferents, dopamine, rasagiline.

INTRODUCTION

SNHLs and the lack of their effective pharmacological treatment

Hearing loss (HL) is the most frequent human sensory deficit. In contrast to its conductive forms, there is no specific drug therapy for sensorineural hearing losses (SNHLs; e.g., ototoxicant drug- and noise-induced HL or presbycusis), except for symptomatic approaches with moderate efficacy. One of the main reasons for the absence of specific tools to prevent and cure SNHLs is the insufficient knowledge of the basic molecular mechanisms of normal and impaired adult hearing and of the endogenous protective factors.

A consensus is evolving that the imbalance of the redox homeostasis and the consequent increase in reactive oxygen and nitrogen species (ROS, RNS) is a common pathological basis in all the acquired forms of SNHLs (Mukherjea et al., 2011), as well as in the many inherited forms (Noben-Trauth and Johnson, 2009). This knowledge initiated testing of different antioxidants and ROS scavengers (Tabuchi et al., 2010; Mukherjea et al., 2011) for the protection of the cells of the organ of Corti and auditory neurons, which are primary targets in SNHLs.

Rasagiline

Rasagiline, a selective propargylamine inhibitor of monoamine oxidase inhibitor (MAO) type B, has been applied to Parkinson's disease in clinical practice (Finberg, 2010). In addition to selectively inhibiting the

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Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; DA, dopamine; EM, electron microscopy; FR, fractional release; Glu, glutamate; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; HL, hearing loss; IHCs, inner hair cells; LOC, lateral olivocochlear; MAO, monoamine oxidase inhibitor; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNHLs, sensorineural hearing losses; VGCC, voltage-gated calcium channel; VGSC, voltage-gated sodium channel.

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dopamine (DA) metabolizing enzyme MAO-B, it also has a cell protective action. It has been shown to protect against neural degeneration (Huang et al., 1999; Speiser et al., 1999; Youdim et al., 2006), oxidative damage and apoptosis (Tabakman et al., 2004; Siderowf and Stern, 2006). These protective effects provide a rational to test its effect in different forms of SNHLs. Furthermore, as an enhancer of DAergic neurotransmission (Weinreb et al., 2010) in the central nervous system, it may also potentiate the release of DA from the lateral olivocochlear (LOC) efferents, which is considered to be a protective feedback pathway of the cochlea (Pujol et al., 1993; Pujol, 1994; Lendvai et al., 2011; Maison et al., 2013).

The cochleoprotective role of DA released from LOC efferent fibers

It has been shown that the excessive release of glutamate (Glu) from inner hair cells (IHCs) in noise-induced HL, presbycusis, cochlear ischemia or aminoglycoside-induced ototoxicity results in the excitotoxic damage of the primary auditory neurons (Duan et al., 2000; Ruel et al., 2007; Tabuchi et al., 2010; Bernarding et al., 2013). LOC efferents, forming axodendritic synapses with the auditory neurons, serve as the effector arm of the auditory neurons – cochlear nucleus – lateral superior olivary complex – cochlea short-loop feedback and provide protection to the auditory neurons against excitotoxicity by releasing DA. DA inhibits the postsynaptic effects of Glu and protects the IHC-afferent nerve synapse (Halmos et al., 2005, 2008; Ruel et al., 2007; Lendvai et al., 2011). Intracochlear application of the D_2/D_3 dopamine receptor agonist piribedil reduced the characteristic electrophysiological and structural changes evoked by acoustic trauma and ischemia (Pujol et al., 1993; d'Aldin et al., 1995a,b; Gil-Loyzaga, 1995), and D_1, D_2 receptor agonists were shown to inhibit the NMDA- and AMPA-induced firing of the primary afferent nerve (Oestreicher et al., 1997). Although drugs acting on the DAergic system have not yet been tested thoroughly, theoretically, any drug able to boost the function of this system could hold preventive or curative promises for SNHLs (Halmos et al., 2005; Lendvai et al., 2011).

Aminoglycoside ototoxicity and its use as a SNHL model

Aminoglycoside antibiotics, which still need to be used in the treatment of certain serious infections caused by aerobic gram-negative bacteria, can induce irreversible HL (Xie et al., 2011). Hair cells, especially the outer hair cells and the IHC ribbon synapse, together with the auditory neurons, are very vulnerable to the administration of aminoglycosides (Ylikoski et al., 1974; Dodson, 1997; Duan et al., 2000; Maruyama et al., 2008; Fransson et al., 2010; Liu et al., 2013). The pivotal role of normal redox state disturbances, generation of ROS and excitotoxic damage of the auditory neurons in the pathomechanism has been shown in several studies (Basile et al., 1996; Sha and Schacht, 1999; Duan et al., 2000; Poirrier et al., 2010; Huth et al., 2011). This serious side effect is the basis of a well-established animal model used in hearing research (Wu et al., 2001). As the aminoglycoside induced HL involves oxidative stress, ROS generation and excitotoxic neuronal damage, we tested the effect of rasagiline in the kanamycin-induced hearing loss model.

EXPERIMENTAL PROCEDURES

In vivo measurement of the rasagiline effect in the aminoglycoside-induced ototoxicity model

General experimental paradigm of kanamycin-induced ototoxicity and application of rasagiline. All animal care and experimental procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Procedures were approved by the Animal Use Committee of the Institute of Experimental Medicine, Hungarian Academy of Sciences. Selections of the mouse strain and the type and concentration of aminoglycoside antibiotic were based on data from the literature (Wu et al., 2001). Our preliminary experiments (data not shown) testing different mouse strains, aminoglycoside antibiotics and concentrations of kanamycin, confirmed that the most pronounced and reliable aminoglycoside-induced hearing loss, suitable for testing otoprotection, could be produced in BALB/c mice by administering kanamycin in an 800 mg/kg s.c. dose. Male BALB/c mice, age 4 weeks, were purchased from Charles River, Germany.

First, a set of experiments exploring also the dynamics of the effect of kanamycin and rasagiline was carried out. Mice were assigned to one of the following four experimental groups: (1) Control (physiological saline), (2) Kanamycin, 800 mg/kg, (3) Rasagiline, 3 mg/kg, and (4) Kanamycin, 800 mg/kg + Rasagiline, 3 mg/kg. Treatment groups contained eight mice each. (One mouse in group 4 died during the auditory brainstem response (ABR) measurement under anesthesia.) Kanamycin sulfate (USB Corporation, Cleveland, OH) was injected s.c. twice daily (8–9 a.m. and 6–7 p.m.) for 2 weeks. The first dose of the antibiotic was administered on the day of the first ABR measurement (6–7 p.m.) after all the measurements had been performed. Doses of rasagiline mesylate (3 mg/kg, s.c.; TEVA) were given once daily at the same time as the morning dose of kanamycin, but the injections were separate. In this way, the first dose of rasagiline was delivered 14 h after the first kanamycin dose. Rasagiline treatments lasted 5 weeks. Mice in the Control group were injected s.c. by an equivalent amount of physiological saline. In the kanamycin treatment group, after the 2nd week, the kanamycin injections were replaced by injections of physiological saline till the end of the 5th week.

Auditory thresholds were determined in both ears from the ABRs. Thresholds were taken from each animal prior to the start of the drug treatments on the 1st week (start-up threshold), 2 weeks after the start of drug treatment, and then weekly up to 5 weeks (5 measurements in sum). The threshold shift gives the difference of an