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Research Report

Effects of substance P during the recovery of hearing function after noise-induced hearing loss

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ABSTRACT

Substance P (SP) is a widely distributed neurotransmitter in living tissues and is involved in various repair processes. We investigated the possibility that SP may ameliorate cochlear hair cell damage produced by noise exposure. The present study examined the effect of SP in protecting the cochlea from noise damage in guinea pigs exposed to noise after an infusion of SP into the inner ear. Changes in the hearing threshold (auditory brain response, ABR), number of synaptic ribbons, and the appearance of the outer hair cells after noise exposure were analyzed at 2 severity levels of noise-induced hearing loss. The moderate noise-induced hearing loss (110 dB, 3 h) group showed recovery in the ABR threshold over time, finally reaching a level slightly above pre-exposure levels, with only slight injury to the synaptic ribbons and minimal changes in the appearance of the outer hair cells. Our results indicated that in moderate hearing loss, SP exhibited a protective effect on the inner ear, both functionally and structurally. While the final magnitude of ABR threshold elevation was greater in severe noise-induced hearing loss, the synaptic ribbons and outer hair cells showed signs of severe damage.

1. Introduction

It is known that exposure to excessive noise can cause mechanical and metabolic disorders of the cochlea (Slepecky, 1986). The hearing threshold elevation after noise exposure is either temporary (temporary threshold shift: TTS) or permanent (permanent threshold shift: PTS) depending on the intensity and duration of noise exposure (Sugahara et al., 2003; Hirose et al., 2008). Substance P (SP), first detected in 1931 by Euler and Gaddum (Euler and Gaddum, 1931), is now known to be distributed extensively as an excitatory neurotransmitter in the central and peripheral nerves (Hökfelt et al., 1977), and the cochlea is known to utilize SP as a neurotransmitter (Nowak et al., 1986a,b; Ylikoski et al., 1989; Igarashi et al., 1992; Usami et al., 1993); however, the exact role of SP in the inner ear is not known. We previously reported that a direct unilateral infusion of SP into the peripheral vestibule induced nystagmus (Orita et al., 2009). The influence of SP on synapse has also shown (Parker and Grillner, 1998). Parker and Grillner reported that the amplitude of signal entry from an axon of a lamprey was reinforced by SP treatment, suggesting the possibility that SP modulates synaptic transmission. To date, there is no report on

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the influence of SP on noise-induced hearing loss. We investigated the possibility that SP may ameliorate cochlear hair cell damage produced by noise exposure. The present study aimed to evaluate the effects of SP in protecting ears from auditory disorders.

In the present study, we created 2 severity levels of noise-induced hearing loss: moderate noise-induced hearing loss (110 dB, 3 h) based on a TTS model, and severe noise-induced hearing loss (130 dB, 3 h), created in a similar fashion to a PTS model. Histologically, moderate hearing loss was expected to involve damage to the synapses beneath the inner hair cells (IHCs) with no damage to the outer hair cells (OHCs), resembling TTS, while severe hearing loss would involve damage to both the IHCs and OHCs, resembling PTS (Spoendlin, 1985; Hu et al., 2006). Functionally, moderate noise-induced hearing loss is expected to show recovery soon after the end of noise exposure, resembling TTS, although unlike TTS, the final hearing threshold remains slightly higher than the pre-exposure level, while in severe hearing loss, the final hearing threshold is expected to be considerably higher than the pre-exposure level. We examined changes in the synaptic ribbons over time beneath the IHC and OHC nuclei after noise exposure and in hearing thresholds before and after noise exposure in both groups. Additionally, we tested the protective effects of various doses of SP applied directly to the cochlea in both groups, as well as the possible role of SP during recovery from noise-induced hearing loss, while focusing on 2 aspects: changes in inner ear function and histological changes.

2. Results

2.1. *Changes in the number of synaptic ribbons at 2 different severity levels of noise-induced hearing loss*

Changes in the number of synaptic ribbons over time beneath the IHC nuclei were analyzed at 2 severity levels of noise-induced hearing loss by counting the number of C-terminal binding protein-2 (CtBP2) signals beneath the immunostained IHC nuclei. As shown in Fig. 1, the distribution of dots positively stained with anti-CtBP2 antibody was identical to the distribution of cochlear nerve endings stained with anti-neurofilament heavy polypeptide antibody. Anti-CtBP2

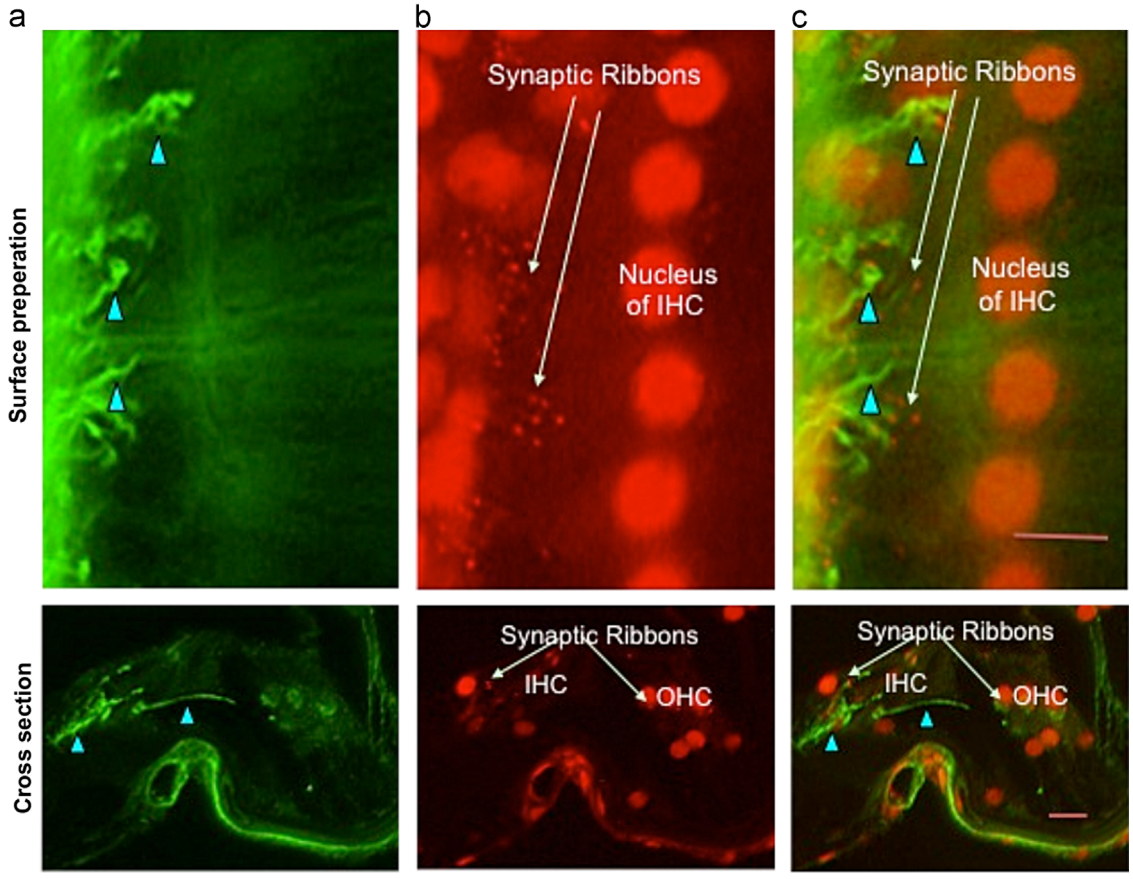

Fig. 1 – Staining of the synaptic ribbons. Chromatic responses of the organ of Corti observed in a surface preparation and cross-section. (a) A cochlear nerve stained with anti-neurofilament heavy polypeptide antibody (arrowhead). (b) Synaptic ribbons stained with anti-C-terminal binding protein-2 antibody (arrow). IHC indicates the inner hair cells and OHC indicates the outer hair cells. Positive chromatic responses were noted in the nuclei of the IHCs and OHCs. (c) Images produced by the merging of images of the cochlear nerves stained with anti-neurofilament heavy polypeptide antibody (arrowhead) and the synaptic ribbons stained with anti-C-terminal binding protein-2 antibody (arrow). IHCs=inner hair cells. OHCs=outer hair cells. Surface preparation scale bar=10 μm. Cross-section scale bar=20 μm.