Short Communication

Dehydroepiandrosterone Sulfate Reduces Acoustic Injury of the Guinea-Pig Cochlea

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Abstract. The present study was performed to determine effects of dehydroepiandrosterone sulfate (DHEAS), a neurosteroid, on acoustic injury. Albino guinea pigs were exposed to a 2 kHz pure tone of 120 or 125 dB sound pressure level for 10 min immediately after intravenous administration of DHEAS. Statistically significant improvement in the compound action potential threshold shifts and in amplitude reduction of distortion-product otoacoustic emissions was observed 1 week after the acoustic overexposure in the animals treated with DHEAS. The present results suggest that DHEAS has a protective effect against acoustic injury of the cochlea.

Keywords: dehydroepiandrosterone sulfate (DHEAS), acoustic injury, cochlea

Dehydroepiandrosterone (DHEA, 3 α -hydroxy-5 α -androst-17-one) and its sulfate ester dehydroepiandrosterone sulfate (DHEAS) are steroids secreted by the adrenal cortex in response to adrenocorticotrophine (ACTH). Evidence accumulated over the last decade has documented that the brain is capable of de novo synthesis of DHEA or DHEAS independent of adrenals (named neurosteroids). DHEA and DHEAS have recently received increased attention because it has been demonstrated that they display antioxidant properties and possess modulatory effects on NMDA, GABA, and sigma receptors and calcium channels (1). Thus, they have protective effects in various brain injuries, including glutamate excitotoxicity and anoxia-reoxygenation (2–4).

Evidence from previous studies suggests that oxidative stress and glutamate excitotoxicity are linked to the etiology of various cochlear injuries including acoustic injury. The protective effects of free radical scavengers and NMDA-receptor antagonists on acoustic injury have been demonstrated by several researchers (5, 6). The purported antioxidative and neuroprotective properties of DHEAS strongly suggest that DHEAS may possess protective effects in various cochlear injuries. However, to our knowledge, the effects of DHEAS on acoustic

injury of the cochlea have never been examined.

It has been demonstrated that damage to outer hair cells (OHCs) is the initial effect of acoustic overstimulation (7). Distortion-product otoacoustic emissions (DPOAEs) are supposed to be closely related to an active process in the organ of Corti and are a useful test of OHC function. In the present study, we investigated the effect of DHEAS on acoustic injury. DPOAEs as well as compound action potential (CAP) were monitored before and after acoustic overexposure.

Studies were performed on albino guinea pigs weighing 250–350 g. The animals were anesthetized with intraperitoneal injection of pentobarbital sodium (28 mg/kg, Nembutal®; Abbott, North Chicago, IL, USA). A catheter was inserted in the external jugular vein for administration of DHEAS. Body temperature was maintained at 37 $\pm$ 1°C using a heating pad. All experimental procedures used in this study were approved by the Animal Experiment Committee at the University of Tsukuba.

CAP was recorded at the bony edge of the round window by an Ag/AgCl electrode (8). The reference electrode was placed on the neck muscles. In order to evoke CAP, tone bursts of pure tones from 2 to 16 kHz (rise and fall times, 1 ms; duration, 10 ms) were delivered in an open field system. Responses were averaged, and the sound pressure level necessary to

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Table 1. Frequencies and levels of f1 and f2

GM (Hz)	f1 (Hz/dB SPL)	f2 (Hz/dB SPL)	2f1 – f2 (Hz)
4000	3650/60	4375/50	2925
6000	5475/50	6575/40	4375
8000	7300/50	8800/40	5800

GM: geometric means.

evoke 10 μ V CAP was defined as the threshold.

Because our recording methods for DPOAEs have been described elsewhere (9), only a brief summary will be given here. Two primary tones were delivered to the emitters contained within a custom-made ear probe. The frequencies and levels of the primary tones are shown in Table 1. DPOAEs were recorded by a microphone (EK-3024; Knowles, Itasca, IL, USA) inside the ear probe and processed by a spectrum analyzer (CF-350; Ono Sokki, Tokyo).

In order to induce acoustic trauma, the left ear was exposed to 2 kHz pure tone of 120 or 125 dB SPL (sound pressure level) through the hollow ear bar for 10 min. DHEAS was purchased from Sigma-Aldrich Japan (Tokyo). The animals were divided into the following 4 groups:

1) DHEAS-given 125 dB group (n = 12): Each animal in this group was intravenously given 0.1 (n = 5) or 1 (n = 7) mg/kg DHEAS just before the acoustic overexposure of 125 dB SPL.

2) Physiological saline solution (PSS)-given 125 dB group (n = 7): Each animal in this group received PSS intravenously just before the acoustic overexposure of 125 dB SPL.

3) DHEAS-given 120 dB group (n = 12): Each animal in this group was intravenously given 0.1 mg/kg (n = 5) or 1 mg/kg (n = 7) DHEAS just before the acoustic overexposure of 120 dB SPL.

4) PSS-given 120 dB group (n = 7): Each animal in this group received PSS intravenously just before the acoustic overexposure of 120 dB SPL.

All data were expressed as the mean $\pm$ S.E.M. Shifts in the CAP thresholds and DPOAE amplitudes from the pre-exposure values were compared between the DHEAS- and the PSS-given groups using two-way ANOVA followed by the Scheffe test. The cochlear functions were evaluated at two time points after acoustic overexposure, specifically immediately after the acoustic overexposure to confirm the equality of acoustic overexposure between the groups and 1 week after the acoustic overexposure to evaluate the ultimate cochlear function (permanent threshold shifts after acoustic overexposure). A *P* value of less than 0.05 was considered statistically significant.

Intravenous administration of 1 mg/kg DHEAS (a maximum dose dissolved in PSS) or 0.1 mg/kg DHEAS itself did not significantly affect the CAP threshold or DPOAE amplitude 10 min or 1 week after the administration (n = 4 in each group, data not shown). The CAP threshold and amplitude of DPOAE were also not affected by 0.1 mg/kg DHEAS (n = 3, two-way ANOVA, data not shown).

Figures 1 and 2 show the CAP threshold shifts and reduction in DPOAE amplitude after the acoustic overexposure of 125 dB SPL, respectively. As shown in Figs. 1A and 2A, DHEAS did not affect shifts in the CAP threshold or DPOAE amplitude immediately after the acoustic overexposure. However, 1 mg/kg DHEAS (n = 7) significantly decreased the CAP threshold shifts (Fig. 1B, two-way ANOVA: *P* < 0.01, Scheffe test: *P* < 0.01 in the 1 mg/kg subgroup) and significantly increased DPOAE amplitude 1 week after the acoustic overexposure (Fig. 2B, two-way ANOVA: *P* < 0.01, Scheffe test: *P* < 0.01 in the 1 mg/kg subgroup), although 0.1 mg/kg DHEAS (n = 5) exhibited no such protective effects.

The results obtained in the 120 dB SPL experiment were essentially the same as those in 125 dB SPL. Namely, DHEAS significantly ameliorated the cochlear dysfunction at 1 mg/kg 1 week after the acoustic overexposure, although 0.1 mg/kg DHEAS (n = 5) did not affect the CAP threshold shifts or DPOAE amplitude (two-way ANOVA and Scheffe test: *P* < 0.01 in the 1 mg/kg subgroup).

Several steroids, including DHEA, pregnenolone, progesterone, and their sulfate esters, can be synthesized within the central nervous system and are named neurosteroids. DHEAS has neurotrophic effects (10, 11) and modulatory functions on NMDA, GABA, and sigma receptors (1). Although DHEAS is a widely studied neurosteroid with multifunctional properties on the brain, effects of DHEAS on the cochlear function have not been fully determined. The present study showed that pretreatment with 1 mg/kg DHEAS significantly reduced the CAP threshold shift 1 week after acoustic overexposure. This finding is consistent with the observation on the cytoprotective effect of DHEAS in the central nervous system (12). To our knowledge, this study demonstrated for the first time that DHEAS exerted protective effects against acoustic injury of the cochlea. In addition, based on the results obtained in the DPOAE testing, DHEAS protected OHCs to alleviate CAP threshold shifts after acoustic injury.

It has been demonstrated that antioxidants and NMDA-receptor antagonists ameliorate acoustic injury of the cochlea (5, 6). Although relatively early production of free radicals in the cochlea after acoustic over-

Fig. 1. Threshold shifts in CAP immediately (A) and 1 week (B) after the acoustic overexposure of 125 dB SPL. Closed circles indicate the PSS group, and squares and open circles indicate 1 and 0.1 mg/kg DHEAS groups, respectively. $**P < 0.01$, as compared with the PSS-group.

Fig. 2. Amplitude reduction in DPOAEs (dB re pre-exposure level) immediately (A) and 1 week (B) after the acoustic exposure of 125 dB SPL. Closed circles and open squares indicate the PSS group and 1 mg/kg DHEAS groups, respectively. $**P < 0.01$, as compared with the PSS-group.

exposure was demonstrated by Ohlemiller et al. (13), the precise time courses of free radical production and NMDA-receptor activation during and after acoustic overexposure have not been made clear. Published data have demonstrated that DHEAS acts to limit lipid peroxidation caused by free radicals (12, 14) and prevents glutamate and NMDA excitotoxicity (2). A receptor for DHEAS has not been definitively isolated yet (15), and up to now, these effects of DHEAS are

considered to be non-genomic effects. Because non-genomic effects of steroids are generally observed within a short period, further studies will be necessary to elucidate the timing of free radical production and NMDA-receptor activation and to illustrate the precise protective mechanism(s) of DHEAS in acoustic injury.

In conclusion, besides the neuroprotective effects of DHEAS, we have shown here that DHEAS is able to protect the cochlea against acoustic injury. Functional

findings have revealed that DHEAS protects OHCs to alleviate acoustic injury.

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