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Sustained Fos expression is observed in the developing brainstem auditory circuits of kanamycin-treated rats

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ABSTRACT

It has been demonstrated that kanamycin treatment during early developmental period induces partial cochlear destruction and enhanced glutamatergic transmission at the medial nucleus of the trapezoid body (MNTB) – the lateral superior olive (LSO) synapses in the superior olivary complex (SOC). As *c-fos* was expected to be expressed in the SOC by kanamycin-induced cochlear damage, the expression of *c-fos* protein (Fos) was investigated using immunohistochemistry in kanamycin-treated rat pups. In the control rat pups less than postnatal (P) day 9 in age, Fos-like immunoreactivity (Fos-IR) was transiently observed in the MNTB and LSO on P6, but disappeared on P9, which reflects a physiologic process. In contrast, in kanamycin-treated rats, Fos-IR was consistently observed through P9. Because a significant increase in terminal uridine deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate-biotin nick-end labeling (TUNEL) and glial fibrillary acidic protein (GFAP) IR was not demonstrated in the MNTB and LSO of kanamycin-treated rats, the increased Fos-IR does not appear to indicate an ongoing pathologic process, but may be related to the increased activity caused by the disturbance in excitatory and inhibitory balance between brainstem auditory circuits.

1. Introduction

It has been established that aminoglycosides suppress cochlear function by damaging sensory hair cells [13,17,41]. However, in developing rats, many studies have demonstrated that susceptibility of the auditory organ to aminoglycosides is limited to a critical period of development. Starting either at birth, 10 or 30 days of age, amikacin treatment (200 mg/kg daily) elicited a substantial threshold shift of auditory brainstem response only in the groups treated from 10 to 20 days [4]. Light microscopic examination of inner ears on day 30 revealed no conspicuous cochlear damage by the kanamycin treatment (400 mg/kg daily) from postnatal (P) day 1 to P8, whereas severe cochlear damage was observed in specimens exposed to kanamycin from P8 to P16 [31]. This critical period is known to be the second postnatal week in rats, and prior to this period the cochlea is relatively less susceptible to aminoglycosides [25,30,32].

In rats, the enhanced glutamate co-release from the medial nucleus of the trapezoid body (MNTB), a brainstem auditory nucleus, has been reported by the treatment with kanamycin (from P3 to P8) (700 mg/kg, subcutaneous injection, twice a day), which induced partial, not total damage of cochlear hair cells [22]. This opens the possibility that aminoglycosides affect the secondary relay auditory nuclei via cochlear damage, even during the less susceptible period to aminoglycosides.

As GABA/glycinergic MNTB-lateral superior olive (LSO) synapses co-release glutamate transiently (<P9) [10] and the MNTB-LSO synapses become inhibitory after the first postnatal week in rats [18,23], the prolonged enhancement of glutamatergic co-transmission at the MNTB-LSO synapses induced by kanamycin treatment may alter the excitatory/inhibitory balance between the cochlear nucleus-LSO and MNTB-LSO synapses, which may lead to increased excitability of the auditory brainstem circuits. Therefore, in the present study, the expression of Fos, the product of proto-oncogene (*c-fos*), was examined in the brainstem auditory nuclei during the early auditory developmental period (<P10), when the ototoxic effect of kanamycin is relatively weak, because Fos has been demonstrated to be a useful marker of neural excitation within the central auditory system [24,28,29,37]. A terminal uridine deoxynucleotidyl transferase-mediated

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2′-deoxyuridine 5′-triphosphate-biotin nick-end labeling (TUNEL) and glial fibrillary acidic protein (GFAP) labeling were done to rule out the possibility that differences in *c-fos* expression could be attributable to effects of kanamycin exposure on apoptosis [5,21,36,38].

2. Materials and methods

2.1. Animals and slice preparations

Pregnant female Sprague-Dawley rats were purchased from a domestic company (Samtako BioKorea, Osan, Korea), and their pups were used. The pups were treated with kanamycin dissolved in distilled water (700 mg/kg, subcutaneously, twice a day) from P3 to P5 or P3 to P8, and the pups were evaluated on P6 or P9. In cochlear ablation studies, pups were treated with kanamycin from P10 to P16, and were evaluated on P22. For the P9 sham operation, control pups were injected with distilled water from P3 to P8. However, P6 pups were not injected with distilled water to rule out the possibility of injection-induced Fos expression. The Dankook University Institutional Animal Care and Use Committee (DUIAC) approved this study.

2.2. Immunohistochemistry

The detailed procedure of immunostaining was described in previous papers [15,22]. The primary antibody for Fos was PC38 anti-c-Fos (Ab-5; 4-17) rabbit pAb (1:2500 dilution; Calbiochem, Damstadt, Germany). The sections were evaluated using an Olympus BX51 microscope (Olympus, Japan). After obtaining images with a microscope digital camera system (DP50; Olympus), we converted the images (JPEG or TIFF format) to black and white images, isolated the darkly-stained Fos-positive cells above the basal background staining intensity by adjusting the intensity threshold, and counted cells automatically with the Image J program (Image J 1.44p; NIH, Bethesda, MD, USA). Only neurons in the MNTB were counted, because we could identify the border relatively clearly. The area of the MNTB was measured with a computer program (DP2-BSW, version 2.1; Olympus). The procedure for the immunofluorescence studies was similar to that described previously [22]. The primary antibodies used were rabbit polyclonal anti-GFAP (diluted to 1:500, AB7260; Abcam, Cambridge, UK) and guinea pig polyclonal anti-VGLUT3 (1:100,000, AB5421; Millipore, CA, USA).

Terminal uridine deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate-biotin nick-end labeling (TUNEL)

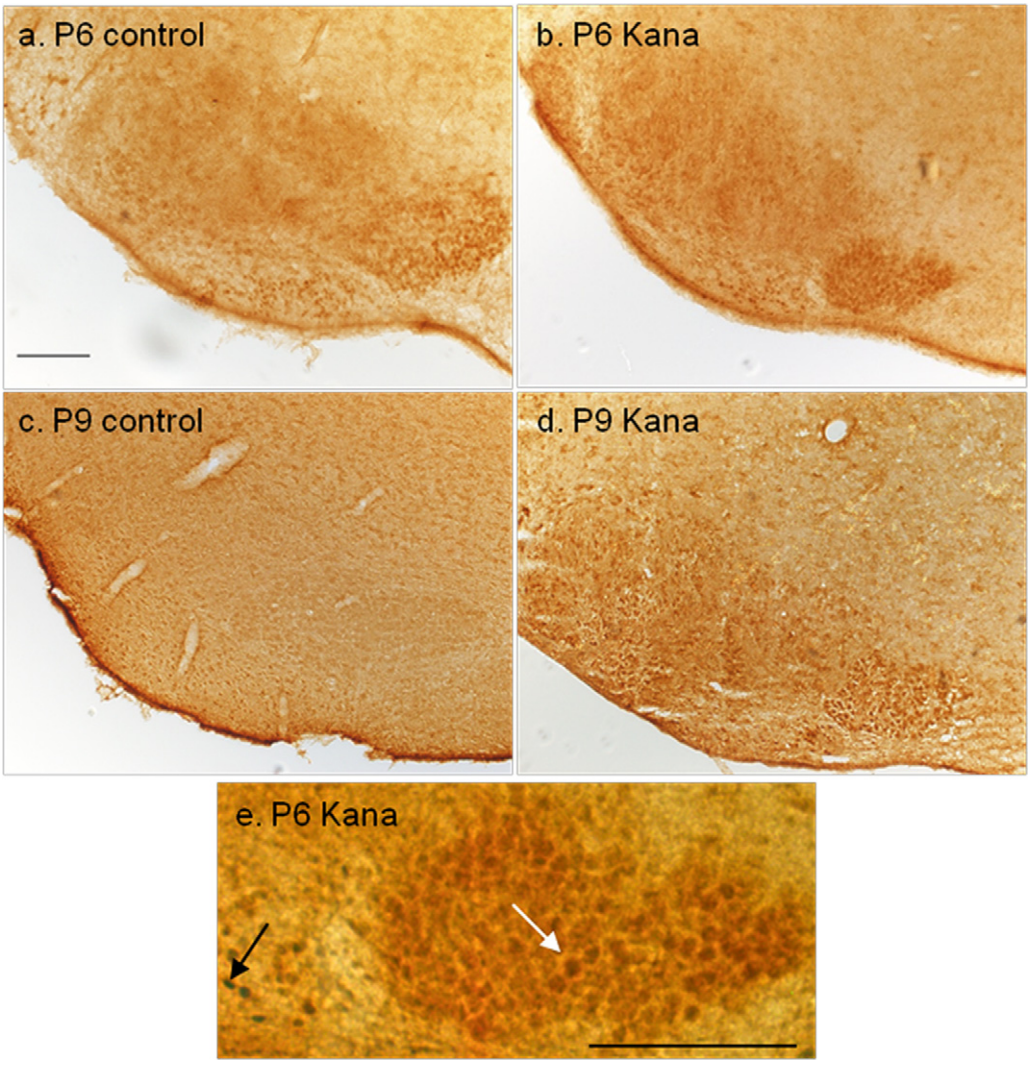

Fig. 1. Fos-IR in the SOC. Fos-IR-positive neurons were observed in the SOC of both P6 control (a) and kanamycin-treated (P6 Kana) rats (b). The similar staining pattern was observed only in P9 kanamycin-treated rats (P9 Kana) (d), but not in P9 control rats (c). MNTB area shown in (b) is magnified in (e). The black arrow indicates nuclear staining, whereas the white arrow indicates both cytoplasmic and nuclear staining. Scale bars in (a) and (e) indicate 200 μm.