Neuroscience 258 (2014) 410–421

EFFECTS OF EARLY AND LATE TREATMENT WITH L-BACLOFEN ON THE DEVELOPMENT AND MAINTENANCE OF TINNITUS CAUSED BY ACOUSTIC TRAUMA IN RATS

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Abstract—Subjective tinnitus is a chronic neurological disorder in which phantom sounds are perceived. Recent evidence supports the hypothesis that tinnitus is related to neuronal hyperactivity in auditory brain regions, and consequently drugs that increase GABAergic neurotransmission in the CNS, such as the $GABA_B$ receptor agonist L-baclofen, may be effective as a treatment. The aim of this study was to investigate the effects of early (5 mg/kg s.c., 30 min and then every 24 h for 5 days following noise exposure) and late treatment (3 mg/kg/day s.c. for 4.5 weeks starting at 17.5 weeks following noise exposure) with L-baclofen on the psychophysical attributes of tinnitus in a conditioned lick suppression model following acoustic trauma in rats. Acoustic trauma (a 16-kHz, 115-dB pure tone presented unilaterally for 1 h) resulted in a significant decrease in the suppression ratio (SR) compared to sham controls in response to 20-kHz tones at 2, 10 and 17.5 weeks post-exposure ($P \leqslant 0.009$, $P \leqslant 0.02$ and $P \leqslant 0.03$, respectively). However, L-baclofen failed to prevent the development of tinnitus when administered during the first 5 days following the acoustic trauma and also failed to reverse it when treatment was carried out every day for 4.5 weeks. We also found that treatment with L-baclofen did not alter the expression of the $GABA_B$-R2 subunit in the cochlear nucleus of noise-exposed animals.

Key words: acoustic trauma, tinnitus, L-baclofen, $GABA_B$ receptors, rats.

INTRODUCTION

Tinnitus is the perception of a ringing, buzzing or whistling sound in the absence of an external sound, which causes sleep disturbances, cognitive problems, work impairment and even suicide (Axelsson and Ringdahl, 1989; Hoffmann and Reed, 2004; Khedr et al., 2010). Tinnitus affects 25% of people in the USA at some stage in their life, with 8% experiencing persistent or chronic tinnitus (Shargorodsky et al., 2010). Furthermore, the incidence of tinnitus is fourfold higher in MP3 player users (Figueiredo et al., 2011). A recent cost study in the Netherlands revealed that the mean annual tinnitus-related health care and societal costs were €10,561 per patient and the estimated total societal cost in the population was €6.8 billion in 2009 (Maes et al., 2013). The burden of tinnitus on an individual's employment status, work-related productivity and healthcare-related costs is likely to increase in the future because many people spend prolonged periods of time listening to loud music through headphones.

At present, there is no reliably effective treatment for tinnitus. Drugs are one of a number of potential treatment avenues; however, to date, there have been few drugs that work consistently to alleviate the condition (see Langguth and Elgoyhen, 2012 for a recent review). Data from the acoustic trauma animal model of tinnitus have suggested that tinnitus is associated with neuronal hyperactivity at different levels of the central auditory pathways, including the dorsal and ventral cochlear nucleus (CN), the inferior colliculus and the auditory cortex (see Kaltenbach, 2006; Rauschecker et al., 2010; Roberts et al., 2010 for reviews; see Dong et al., 2010; Middleton et al., 2011; Mulders and Robertson, 2011; Vogler et al., 2011; Gu et al., 2010; Pilati et al., 2012; Manzoor et al., 2013a,b; Robertson et al., 2013, for recent examples). Functional MRI studies have also indicated that humans with tinnitus exhibit hyperactivity in the auditory cortex and ventral striatum (Gu et al., 2010; Leaver et al., 2011). These data support the hypothesis that tinnitus may be a form of sensory epilepsy (Moller, 2000). Although the mechanisms underlying the tinnitus-related neuronal hyperactivity are not entirely understood, one possibility is a decrease in synaptic inhibition mediated by neurotransmitters such as GABA and glycine (Middleton et al., 2011; see Wang et al., 2011 and Richardson et al., 2012 for recent reviews). Therefore, drugs that increase synaptic inhibition, such as agonists at the $GABA_A$ (e.g., benzodiazepines) and $GABA_B$ receptors (e.g., L-baclofen), have been investigated as potential treatments for tinnitus.

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Abbreviations: ABR, auditory brainstem-evoked response; ANOVA, analysis of variance; BBN, broad band noise; CS, conditioned stimulus; CN, cochlear nucleus; GAD-67, glutamic-acid decarboxylase-67; LMM, linear mixed model; NMDA, *N*-methyl-D-aspartate; SPL, sound pressure level; SR, suppression ratio.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.11.032

The anti-spasticity agent and $GABA_B$ receptor agonist, L-baclofen, was demonstrated to have greater efficacy than some benzodiazepines in reducing tone- and click-evoked neuronal activity in the inferior colliculus (Szczepaniak and Møller, 1995, 1996), suggesting the possibility that a down-regulation of $GABA_B$ receptors could be implicated in the neuronal hyperactivity underlying tinnitus. However, in the only published clinical trial of baclofen in patients, while the subjective ratings of tinnitus were significantly reduced following drug administration compared to before treatment, there was no significant difference compared to the placebo group (Westerberg et al., 1996). Nonetheless, Westerberg et al. (1996) apparently used racemic baclofen, a mixture of L- and D-baclofen, which has been reported to be less potent than L-baclofen alone (Szczepaniak and Møller, 1995; Smith et al., 2012). In an acoustic trauma model of tinnitus using rats, we demonstrated that L-baclofen could reverse the psychophysical indices of tinnitus in a dose-dependent manner (Zheng et al., 2012b). However, it was noticed that once the drug was eliminated from the system, tinnitus recurred (Zheng et al., 2012b). These results suggest that although L-baclofen might be effective in modulating noise-induced tinnitus by reducing neuronal hyperactivity through increasing GABAergic neurotransmission, it cannot permanently reverse the neuronal hyperactivity that has already developed after the noise trauma. We hypothesize that noise trauma may result in a reduction in GABAergic neurotransmission in the CN, causing neuronal hyperactivity and tinnitus, as suggested by previous studies (Dong et al., 2009; Middleton et al., 2011; Browne et al., 2012; Brozoski et al., 2012), and that enhancing this inhibitory synaptic transmission by administering a $GABA_B$ receptor agonist, may therefore reduce tinnitus. Therefore, this study aimed to prevent the development of neuronal hyperactivity, and hence prevent the development of tinnitus, by administering L-baclofen at early time points following the noise trauma.

EXPERIMENTAL PROCEDURES

Subjects

Data were obtained from 32 male Wistar rats (300–350 g at the beginning of the study) divided into four groups: noise-exposed + vehicle ($n = 8$): noise-exposed + L-baclofen ($n = 8$): non-exposed (sham) + vehicle ($n = 8$): and non-exposed (sham) + L-baclofen ($n = 8$). The animals were maintained on a 12:12-h light:dark cycle at 22 °C and were given free access to food, but were water deprived throughout the tinnitus behavioral test. All procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals.

Drug administration

L-Baclofen (R(+)-baclofen hydrochloride, Sigma, G013) was dissolved in saline and each rat received treatment with vehicle or L-baclofen (5 mg/kg, s.c.) 30 min after the noise (or sham) exposure (see below) and then again every 24 h for 5 days. The animals were then tested for the behavioral signs of tinnitus at 2 weeks, and then again at 10 weeks, following noise (or sham) exposure. In addition, beginning at 17.5 weeks after the noise (or sham) exposure, and throughout a third tinnitus testing period, L-baclofen was again administered at a dose of 3 mg/kg/day s.c. for 4.5 weeks. The dose was lowered in this latter part of the study due to adverse effects (e.g., sedation) observed in response to the 5-mg/kg dose. Therefore, L-baclofen was administered: (1) following the noise or sham exposure, for the first 5 days; and (2) at 17.5 weeks following the noise or sham exposure, for 4.5 weeks.

Noise trauma to induce chronic tinnitus in rats

Unilateral acoustic trauma was delivered using a procedure described previously (Bauer and Brozoski, 2001; Brozoski et al., 2007; Zheng et al., 2011a,b,c, 2012a,b). Briefly, the animals were anesthetized with fentanyl citrate (0.2 mg/kg, s.c.), medetomidine hydrochloride (Domitor, Novartis, Mississauga, Ontario, Canada; 0.5 mg/kg, s.c.), and atropine sulfate (50 μg/kg, s.c.), and were placed inside a sound-attenuation chamber for a 1-h exposure to a 16-kHz, 115-dB sound pressure level (SPL) pure tone delivered to one of the ears as described in detail previously (Zheng et al., 2011a,b,c, 2012a,b). This kind of stimulus has previously been reported to induce tinnitus (Tan et al., 2007; Zheng et al., 2011a,b,c, 2012a,b). Acoustic values were calibrated before noise exposure by connecting the speaker to a ¼-inch prepolarized free-field microphone (Type 40BE, GRAS Sound & Vibration) via the speculum used to fit into the external auditory canal. The unexposed ear was blocked with cone-shaped foam and taped against the foam surface. The sham animals were kept under anesthesia for the same duration as the noise trauma animals, but without noise exposure.

Auditory brainstem responses

Auditory function in both ears of exposed and sham animals before and immediately after the acoustic trauma or sham treatment, and at the end of the experiment, were measured using auditory brainstem-evoked response (ABR) thresholds described previously (Zheng et al., 2011a,b,c, 2012a,b). Briefly, the animals were anesthetized as previously described and subdermal needle electrodes were placed at the vertex and over the bullae with a reference electrode at the occiput. Tone bursts of 5-ms duration (2-ms rise/decay, 1-ms plateau) presented at a rate of 21/sec were used to test ABR thresholds. They were presented in a series of decreasing intensity, which began at a level that resulted in distinct evoked potentials. Intensities progressed in 20-, 10-, and 5-dB steps, at 32-, 20-, 16-, and 8-kHz, and the ABR threshold was taken as the lowest intensity that produced a visually distinct potential.