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A dose–response analysis of the effects of L-baclofen on chronic tinnitus caused by acoustic trauma in rats

Yiwen Zheng [a,b], Shweta Vagal [a,b], Emily McNamara [a,b], Cynthia L. Darlington [a,b,1], Paul F. Smith [a,b,*,1]

[a] Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand
[b] Brain Health Research Centre, University of Otago, Dunedin, New Zealand

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ABSTRACT

Subjective tinnitus is a chronic neurological disorder in which phantom sounds are perceived. Drugs that increase GABAergic neurotransmission in the CNS are sometimes used as a treatment. One such drug is the $GABA_B$ receptor agonist L-baclofen. The aim of this study was to investigate the effects of L-baclofen on the psychophysical attributes of tinnitus in rats.The effects of 1, 3 or 5 mg/kg L-baclofen (s.c.) on the psychophysical attributes of tinnitus were investigated using a conditioned lick suppression model, following acoustic trauma (a 16 kHz, 110 dB pure tone presented unilaterally for 1 h) in rats. In pre-drug testing, acoustic trauma resulted in a significant increase in the auditory brainstem-evoked response (ABR) threshold in the affected ear ($P < 0.008$) and a significant decrease in the suppression ratio (SR) compared to sham controls in response to the 20 kHz tones, but not the broadband noise or the 10 kHz tones ($P < 0.002$). The 3 and 5 mg/kg doses of L-baclofen significantly reversed the frequency-specific decrease in the SR in the acoustic trauma group, indicating that the drug reduced tinnitus. Following washout from the 3 mg/kg dose, but not the 5 mg/kg dose, the significant decrease in the SR for the acoustic trauma group returned, suggesting a return of the tinnitus. These results suggest that L-baclofen should be reconsidered as a drug treatment for tinnitus.

This article is part of a Special Issue entitled 'Post-Traumatic Stress Disorder'.

1. Introduction

Subjective tinnitus is a phantom sensation of sound, which has been estimated to occur in 25% of people in the USA, with 8% experiencing it frequently (Shargorodsky et al., 2010). Drugs are one of a number of potential treatment avenues for tinnitus; however, to date, there have been few drugs that work reliably to alleviate the condition (see Dobie, 1999; Darlington and Smith, 2007; Hoekstra et al., 2011, for reviews).

Data from animal models, particularly the acoustic trauma model, have indicated that tinnitus is associated with neuronal hyperactivity at different levels of the central auditory pathways, including the dorsal cochlear nucleus, the inferior colliculus and the auditory cortex (see Refs. Eggermont and Roberts, 2004; Eggermont, 2005; Kaltenbach, 2006; Rauschecker et al., 2010; Roberts et al., 2010 for reviews; see Refs. Dong et al., 2010a,b; Vogler et al., 2011; Middleton et al., 2011 and Mulders and Robertson, 2011 for recent examples). Recent functional MRI studies have also indicated that humans with tinnitus exhibit hyperactivity in the auditory cortex and ventral striatum (Leaver et al., 2011). These data are consistent with the hypothesis that tinnitus is a form of sensory epilepsy (Moller, 2000). The mechanism underlying the neuronal hyperactivity associated with tinnitus is not entirely understood, but one possibility is a decrease in synaptic inhibition normally mediated by neurotransmitters such as GABA and glycine (Middleton et al., 2011; Wang et al., 2011). Consequently, drugs that increase synaptic inhibition, such as benzodiazepines and $GABA_B$ receptor agonists, have been one avenue of investigation for potential new treatments for tinnitus.

Animal studies in the 1990's indicated that the $GABA_B$ receptor agonist, L-baclofen, had greater efficacy than some benzodiazepines in reducing tone- and click-evoked hyperexcitability of neurons in the inferior colliculus (Szczepaniak and Møller, 1995, 1996), which suggested the possibility that a down-regulation of $GABA_B$ receptors could be specifically implicated in the mechanisms of the neuronal hyperactivity underlying tinnitus. However, the only published clinical trial of baclofen in patients with tinnitus yielded inconsistent results. While their subjective ratings of

Abbreviations: SR, suppression ratio; ABR, auditory brainstem evoked response; SPL, sound pressure level; BBN, broad band noise.

* Corresponding author. Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand. Tel.: +64 3 4795747; fax: +64 3 4799140.
E-mail address: paul.smith@stonebow.otago.ac.nz (P.F. Smith).

[1] These authors contributed equally to this work.

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tinnitus were significantly reduced following drug administration compared to before drug administration, there was no significant difference compared to the placebo group (Westerberg et al., 1996). Despite these results, interest in the possible use of L-baclofen to treat tinnitus has remained, with some commentators pointing out that the study was potentially underpowered statistically (Møller, 1997), a possibility also acknowledged by the authors themselves (Westerberg et al., 1996). Furthermore, Westerberg et al. (1996) apparently used racemic baclofen, which has been reported to be less potent than L-baclofen (Szczepaniak and Møller, 1995).

We sought to re-investigate the potential of L-baclofen to treat tinnitus using an acoustic trauma animal model in which tinnitus was indicated by a reduced suppression ratio in a conditioned lick suppression paradigm (Zheng et al., 2011a,b,c).

2. Methods

2.1. Subjects

Data were obtained from 16 male Wistar rats (300–350 g at the beginning of the study) divided into acoustic trauma ($n = 8$) and sham control ($n = 8$) groups. The animals were maintained on a 12:12 h light:dark cycle at 22 °C and were water deprived throughout the tinnitus behavioural test. All procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals.

2.2. Drug administration

L-baclofen (synthesised as R(+)-β-(Aminomethyl)-4-chlorobenzenepropanoic acid hydrochloride, Sigma-Aldrich, St Louis, catalogue no: G013, the more active enantiomer of baclofen) was dissolved in saline and animals received a single vehicle, or 1, 3 or 5 mg/kg s.c injection 1 h before being tested with the conditioned lick suppression paradigm (see below), using a counterbalanced design, so that all animals received all treatments. These drug doses were chosen based on pilot experiments. Animals were first tested in the paradigm at 2 weeks following the acoustic trauma to confirm the presence of tinnitus and then tested following the vehicle, 1, 3 and 5 mg/kg L-baclofen treatments; as well as during the washout between the 3 and 5 mg/kg L-baclofen treatments and following the washout of the 5 mg/kg L-baclofen treatment.

2.3. 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). Briefly, the animals were anaesthetised with ketamine HCl (75 mg/kg, s.c.) and medetomidine hydrochloride (0.3 mg/kg, s.c.) and were placed inside a sound attenuation chamber for a 1 h exposure to a 16 kHz 110 dB SPL pure tone delivered to one of the ears as described in detail previously (Zheng et al., 2011a,b,c).

A 16 kHz, 110 dB pure tone generated by an NI 4461 Dynamic Signal Acquisition and Generation system (National Instruments New Zealand Ltd) was delivered to one of the ears through a closed field magnetic speaker with a tapered tip (Tucker-Davis Technologies), attached to a 3-mm cone-shaped speculum that was fitted tightly into the external auditory canal, for 1 h. This kind of stimulus has previously been reported to induce tinnitus (Tan et al., 2007; Zheng et al., 2011a). Sound pressures were calibrated before noise exposure by connecting the speaker to a ¼-inch prepolarised free-field microphone (Type 40BE, GRAS Sound & Vibration) via the speculum used to fit into the external auditory canal. Since these calibrations were not performed *in situ* with a probe tube microphone in the animal, they may not have accurately reflected sound pressures at the eardrum. The unexposed ear was blocked with cone-shaped foam and taped against the foam surface. The sham animals were kept under anaesthesia for the same duration as the noise trauma animals, but without noise exposure.

2.4. Auditory function

Auditory function in both ears of exposed and sham animals before and immediately after the acoustic trauma or sham treatment was measured using auditory brainstem-evoked response (ABR) thresholds described previously (Zheng et al., 2011a). Briefly, the animals were anaesthetised as previously described and subdermal needle electrodes were placed at the vertex and over the bullae with a reference electrode at the occiput. ABR thresholds were tested for tone bursts (2 ms rise/decay, 1 ms plateau) presented at a rate of 50/s, in a decreasing intensity series, beginning with levels that elicited distinct evoked potentials. Hearing threshold was indicated by the lowest intensity that produced visually distinct potentials.

2.5. Tinnitus assessment

The presence of tinnitus was assessed in each rat after the acoustic trauma using a conditioned lick suppression paradigm described in our previous publications (Zheng et al., 2011a,b,c). Tinnitus assessment was conducted in an operant conditioning test chamber (ENV-007, Med Associates Inc.) using a conditioned lick suppression paradigm, which we developed based on the lever pressing paradigm described by Brozoski et al. (2007). Drinking activity was measured by a lickometer with a photobeam (ENV-251L, Med Associates Inc.). A speaker (ENV-224DM, Med Associates Inc.) directly above the drinking tube produced broadband noise (BBN) or a pure tone of different frequencies and intensities via a sound generator (ANL926, Med Associates Inc.). The BBN was white noise ranging from 3 kHz to 20 kHz with no equalization and the level was measured using an NI 4461 Dynamic Signal Acquisition and Generation card (National Instruments New Zealand) and was calibrated as dB (SPL). The chamber floor was lined with stainless steel rods (0.48 cm in

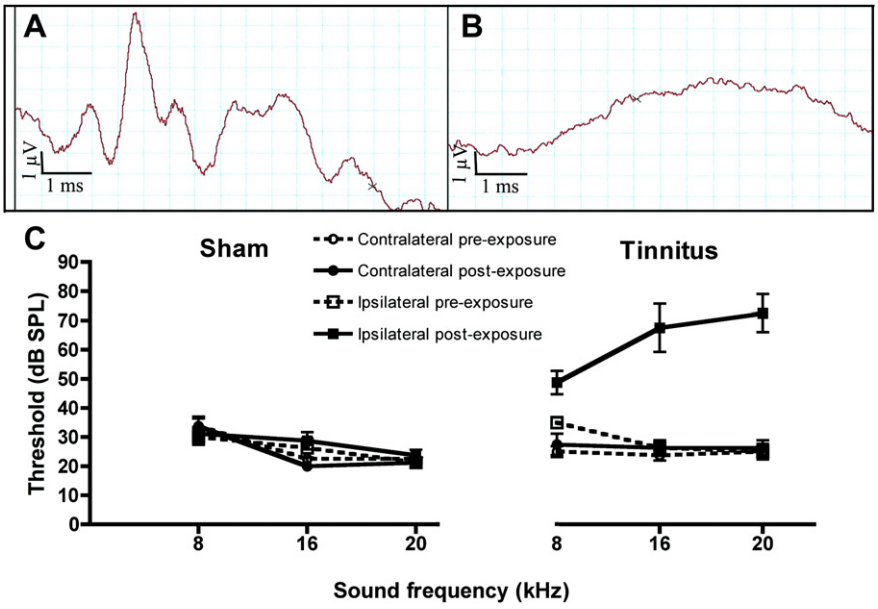

Fig. 1. Examples of ABR recordings (A: pre-exposure and B: post-exposure recorded in response to a 20 kHz, 60 dB SPL tone) and ABR thresholds (C) for the ipsilateral and contralateral ears of acoustic trauma-exposed and sham control animals before and after exposure, as a function of intensity in dB SPL and frequency in kHz. Bars represent means +1 SE.