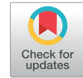
Hearing Research 361 (2018) 36–44

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Effects of AUT00063, a Kv3.1 channel modulator, on noise-induced hyperactivity in the dorsal cochlear nucleus



Lyall Glait [a], Weiwen Fan [a], Gina Stillitano [a], Sharon Sandridge [a], Nadia Pilati [b], Charles Large [c], Giuseppe Alvaro [c], James A. Kaltenbach [a,*]

[a] Department of Neurosciences, Lerner Research Institute, Head and Neck Institute, Cleveland Clinic, USA
[b] Autifony SRL, Via Ugo Bassi 58b, Universita di Padova, 35131, Padova, Italy
[c] Autifony Therapeutics Limited, Stevenage, Biosciences Catalyst, Gunnels Wood Road, Stevenage, SG1 2FX, UK

ARTICLE INFO

Article history:
Received 6 June 2017
Received in revised form
21 December 2017
Accepted 31 January 2018
Available online 7 February 2018

Keywords:
Tinnitus
Hyperactivity
Spontaneous activity
AUT00063
Tinnitus therapy

ABSTRACT

The purpose of this study was to test whether a Kv3 potassium channel modulator, AUT00063, has therapeutic potential for reversing noise-induced increases in spontaneous neural activity, a state that is widely believed to underlie noise-induced tinnitus. Recordings were conducted in noise exposed and control hamsters from dorsal cochlear nucleus (DCN) fusiform cells before and following intraperitoneal administration of AUT00063 (30 mg/kg). Fusiform cell spontaneous activity was increased in sound-exposed animals, approximating levels that were nearly 50% above those of controls. Administration of AUT00063 resulted in a powerful suppression of this hyperactivity. The first signs of this suppression began 13 min after AUT00063 administration, but activity continued to decline gradually until reaching a floor level which was approximately 60% of pre-drug baseline by 25 min after drug treatment. A similar suppressive effect of AUT00063 was observed in control animals, with onset of suppression first apparent at 13 min post-treatment, but continuing to decline toward a floor level that was 54% of pre-drug baseline and was reached 28 min after drug treatment. In contrast, no suppression of spontaneous activity was observed in animals given similar injections of vehicle (control) solution. The suppressive effect of AUT00063 was achieved without significantly altering heart rate and with minimal effects on response thresholds, supporting the interpretation that the reductions of hyperactivity were not a secondary consequence of a more general physiological suppression of the brain or auditory system. These findings suggest that Kv3 channel modulation may be an effective approach to suppressing spontaneous activity in the auditory system and may provide a future avenue for treatment of tinnitus resulting from exposure to intense sound.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Numerous studies have shown that manipulations which cause tinnitus, such as intense sound exposure or treatment with ototoxic drugs (e.g., platin drugs, salicylate) result in the induction of hyperactivity in central auditory centers (Roberts et al., 2010; Kaltenbach, 2011). One major source of this hyperactivity is the fusiform cell population of the dorsal cochlear nucleus (DCN) (Brozoski et al., 2002; Shore et al., 2008; Finlayson and Kaltenbach, 2009; Pilati et al., 2012). The hyperactive state of DCN fusiform cells following noise exposure is associated with and/or may be due in large part to the increased incidence of bursting activity (Finlayson and Kaltenbach, 2009; Bauer et al., 2008; Kalappa et al., 2014; Wu et al., 2016).

A recent study by Pilati et al. (2012) showed that noise-induced increases in bursting activity also occur in DCN fusiform cells in vitro. This bursting activity was associated with increased instantaneous firing of fusiform cells and was associated with a down-regulation of high voltage activated potassium channels with Kv3-like properties. Because bursting activity contributes to increases in spontaneous activity (Finlayson and Kaltenbach, 2009), activation of Kv3 channels may offer a means of reducing the increases in spontaneous activity and are thus attractive as potential therapeutic targets for tinnitus treatment.

AUT00063 (Autifony Therapeutics, Limited) is a small molecule

* Corresponding author. Department of Neurosciences, NE-63, 9500 Euclid Avenue, The Cleveland Clinic Cleveland, Ohio, 44195, USA.
E-mail address: kaltenj@ccf.org (J.A. Kaltenbach).

https://doi.org/10.1016/j.heares.2018.01.017
0378-5955/© 2018 Elsevier B.V. All rights reserved.

modulator of Kv3 ion channels (Anderson et al., accompanying paper). The effects of Kv3 channel modulators have been recently characterized in *vitro* on brainstem neurons (Brown et al., 2016). Briefly, AUT00063 produced a leftward shift in the voltage dependence of activation of Kv3.1 channels and slowed the rate of deactivation of Kv3.1 currents (Anderson et al., accompanying paper). AUT00063 also produced to a lesser extent a leftward shift in the voltage dependence of activation of Kv3.3 channels (Anderson et al., accompanying paper). Both Kv3.3 and Kv3.1 channels are present in the DCN. Consequently, in the presence of AUT00063, Kv3.1 and perhaps Kv3.3 channels will activate earlier during the rising phase of an action potential and deactivate later during the action potential repolarization. This is expected to alter the firing properties of Fc neurons. We hypothesize that the increase in spontaneous firing and bursting activity observed in Fc neurons following noise exposure (Pilati et al., 2012) may be in part due to a reduction in Kv3 current. Thus treatment with AUT00063 may reverse this deficit and reduce the spontaneous activity of DCN Fc neurons, *in vivo*.

Thus the purpose of the work undertaken here was to test the hypothesis that the Kv3 channel modulator, AUT00063, will lead to a reduction of tinnitus-related hyperactivity of DCN fusiform cells, *in vivo*. To test this hypothesis, we induced hyperactivity in DCN fusiform cells of hamsters using intense sound exposure, as described previously (Finlayson and Kaltenbach, 2009). We then quantified changes in spontaneous activity following administration of AUT00063.

2. Methodology

2.1. Animal subjects

Syrian golden hamsters were chosen for this study because previous evidence has been presented that in this species, noise exposure induces a robust increase in spontaneous activity in the dorsal cochlear nucleus (Kaltenbach et al., 2000; Manzoor et al., 2012) that is due in large part to the increase in bursting activity (Finlayson and Kaltenbach, 2009) and is correlated with behavioral evidence of tinnitus (Kaltenbach et al., 2004). Male hamsters were obtained from Charles River in the age range of 60–70 days (weight 100–150 g). Upon arrival, they were placed in a vivarium for at least a 1 week quarantine period. The experiment required three groups of animals, two of which would be sound exposed and one serving as unexposed controls. The exposed animals included a subgroup for testing the effect of AUT00063 and a second subgroup for testing the effect of the vehicle solution. The control group was not exposed to sound but was treated with AUT00063. The Institutional Animal Care and Use Committee of the Cleveland Clinic approved all animal procedures in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Experiment design

Each group of animals was individually studied over a 6–8 week period that began with sound exposures, followed 4–6 weeks later by recordings of the auditory brainstem responses (ABR) to assess effects of sound exposure on auditory sensitivity. Within a few days of ABR recordings, each animal was then studied electrophysiologically, during which surgical exposure of the left DCN followed by placement of the microelectrode array to record spontaneous and stimulus-evoked activity (response areas) was performed. Generally, the electrophysiological recording session ranged between 2 and 3 h. During this period, frequency tuning was tested by recording response areas (see below), and spontaneous activity was measured in successive 5 s epochs for 50 min, which included 1000 s of pre-drug baseline spontaneous activity, followed by a 2000 s recording of post-drug spontaneous activity. Heart rate was monitored during the pre- and post-drug periods. At the completion of the spontaneous activity recording, frequency tuning was again assessed. Although the actual time to perform the surgery and collect these measures was only about 2 h, in some animals, additional time was required to find electrode locations where activity was sufficiently stable to permit study of the drug effect.

2.3. Sound exposure

The exposure to sound was designed to induce hyperactivity in the DCN. We exposed the animals to a 10 kHz tone at an intensity of 115 dB SPL for 4 h. Animals were exposed in groups of 4, as in previous studies (Finlayson and Kaltenbach, 2009; Manzoor et al., 2013a, 2013b). The exposure tone was delivered from a 6 inch loudspeaker (Beyma) driven by a Sherwood RX-4109 amplifier. At the end of the exposure period, the animals were returned to the vivarium and allowed a post-exposure recovery period of 4–6 weeks.

2.4. Auditory brainstem response (ABR)

A few days before beginning drug tests, thresholds were assessed by measuring the ABRs. These were performed in ketamine/xylazine anesthetized animals using an IHS system as described in our recent publication (Chen et al., 2013). Electrode signals were amplified 100,000X and digitally bandpass filtered. Stimuli were presented at a rate of 17.7 stimuli/s, and responses were averaged over 250 stimulus repetitions over a time epoch of 12 ms for clicks and 26 ms for tone pips, including a 1 ms prestimulus period. Stimuli were delivered binaurally through ER2 ear inserts. Stimuli were presented initially at 80 dB SPL then lowered in 20 dB steps until no responses were recorded. This procedure was repeated at each of 4 test frequencies (4, 8, 12, 16 kHz). At each frequency, intensity was bracketed in 5 dB steps to determine threshold at each frequency for each animal.

2.5. Animal surgery

Surgical procedures were identical to those described in our previous studies (Manzoor et al., 2012). In addition, each animal was connected to a CWE Cardio-tachometer (CT-1000) using three subcutaneous electrodes, one each connected to the right and left forepaws, and one to the right hindpaw, These were used to monitor heart rate throughout the recording session to capture any untoward effects of the drug on heart function.

2.6. Recordings of response areas (tuning curves)

The electrophysiological phase of each experiment began with recordings of frequency-intensity response areas at the DCN surface to determine the location of neurons with characteristic frequencies (CFs) of 8–12 kHz. This region was targeted for study because it is here where spontaneous activity has been observed to be maximally elevated following exposure to the tone conditions used in the present study (Manzoor et al., 2012, 2013a, 2013b). The methods for recording response areas were the same as described previously (Manzoor et al., 2012, 2013a, 2013b) except that here response areas were recorded on each of 4 channels of a multielectrode array (FHC) connected to a Plexon model PBX2/16wb multichannel amplifier. Once the location of the 8–12 kHz region of the DCN was determined, the electrode array was lowered to the fusiform cell layer, estimated to be between 200 and 300 μm below the DCN surface (Manzoor et al., 2012). At this depth, we performed

Download English Version:

https://daneshyari.com/en/article/8842387

Download Persian Version:

https://daneshyari.com/article/8842387

Daneshyari.com