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Research paper

Effects of sodium salicylate on spontaneous and evoked spike rate in the dorsal cochlear nucleus

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ABSTRACT

Spontaneous hyperactivity in the dorsal cochlear nucleus (DCN), particularly in fusiform cells, has been proposed as a neural generator of tinnitus. To determine if sodium salicylate, a reliable tinnitus inducer, could evoke hyperactivity in the DCN, we measured the spontaneous and depolarization-evoked spike rate in fusiform and cartwheel cells during salicylate superfusion. Five minute treatment with 1.4 mM salicylate suppressed spontaneous and evoked firing in fusiform cells; this decrease partially recovered after salicylate washout. Less suppression and greater recovery occurred with 3 min treatment using 1.4 mM salicylate. In contrast, salicylate had no effect on the spontaneous or evoked firing of cartwheel cells indicating that salicylate's suppressive effects are specific to fusiform cells. To determine if salicylate's suppressive effects were a consequence of increased synaptic inhibition, spontaneous inhibitory postsynaptic currents (IPSC) were measured during salicylate treatment. Salicylate unexpectedly reduced IPSC thereby ruling out increased inhibition as a mechanism to explain the depressed firing rates in fusiform cells. The salicylate-induced suppression of fusiform spike rate apparently arises from unidentified changes in the cell's intrinsic excitability.

1. Introduction

Hearing loss is often accompanied by tinnitus, a phantom auditory sensation whose severity varies from mild to severe. Among adults, the prevalence of tinnitus ranges from 8 to 15% (Coles, 1984; Henry et al., 2005; Hoffman and Reed, 2004; Nondahl et al., 2002; Snow, 1995) and for approximately 1% the symptoms are severe enough to require medical treatment (Davis and Refaie, 2000; Leske, 1981; Surveys, 1983). While many attempts have been made to identify pharmacological treatments for tinnitus, most drugs have proved ineffective (Dobie, 2004; Salvi et al., 2009). The development of effective drug therapies has been hindered by a poor understanding of the biological bases of tinnitus.

Although many sites along the auditory pathway have been implicated in tinnitus (Basta and Ernst, 2004; Basta et al., 2008; Chen and Jastreboff, 1995; Eggermont and Kenmochi, 1998; Kenmochi and Eggermont, 1997; Llinas et al., 1999; Lockwood et al., 1998; Ma et al., 2006; Mahlke and Wallhausser-Franke, 2004; Sun et al., 2009; Weisz et al., 2007; Zhang et al., 2003), several lines of evidence suggest that the dorsal cochlear nucleus (DCN) plays a key role in its generation (Brozoski et al., 2002; Kaltenbach and Godfrey, 2008; Shore et al., 2007). Acoustic overstimulation, one of the most frequent causes of tinnitus, elevates spontaneous rates in tonotopic regions of the DCN associated with hearing loss (Axelsson and Ringdahl, 1989; Kaltenbach and McCaslin, 1996). The spectral profile and magnitude of the spontaneous rate increase is correlated with behavioral measures of tinnitus (Kaltenbach et al., 2004; Kaltenbach et al., 1998). Since acoustic trauma generally depresses spontaneous activity in the auditory nerve, the hyperactivity observed in the DCN does not appear to originate in the cochlea (Liberman and Dodds, 1984). Moreover, DCN hyperactivity persists after cochlear ablation reinforcing the notion that the hyperactivity is not of cochlear origin (Zacharek et al., 2002). DCN hyperactivity is correlated with the amount of outer hair cell damage; however, the hyperactivity tends to be less when both inner and outer hair cells are damaged (Kaltenbach et al., 2002).

More recent experiments have linked noise-induced tinnitus with hyperactivity in DCN fusiform cells that have best frequencies tuned to the pitch of the tinnitus (Brozoski et al., 2002). Fusiform cells might represent a specific cell type involved in tinnitus

Abbreviations: DCN, dorsal cochlear nucleus; IPSC, inhibitory postsynaptic current; HG-ACSF, high glucose artificial cerebral spinal fluid; SS, sodium salicylate.

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initiation and a pharmacologic target for drug therapy. Fusiform cells receive auditory inputs from the cochlea via auditory nerve fibers as well as vestibular, somatosensory and higher order auditory inputs via parallel fibers originating from granule cells (Golding and Oertel, 1997; Oertel and Young, 2004). Fusiform cells receive glycinergic inhibitory inputs from cartwheel cells. Cartwheel cells receive inputs from parallel fibers and form synapses on other cartwheel cells, giant cells and fusiform cells. Fusiform cells relay their output to the inferior colliculus through dorsal acoustic stria.

High doses of sodium salicylate, the active ingredient in aspirin, induce temporary tinnitus in humans and this effect has been exploited in animal models to investigate the neural correlates of tinnitus at different sites along the auditory pathway (Basta et al., 2008; Lobarinas et al., 2004; Lobarinas et al., 2006; Myers and Bernstein, 1965; Yang et al., 2007). In a brain slice preparation of the cochlear nucleus, salicylate treatment caused spontaneous activity to increase in roughly a third of the units; decrease in another third and had no effect on the remaining third (Basta et al., 2008); however, no information was provided on the changes that occurred in specific cell types or region of the cochlear nucleus where the changes occurred. If tinnitus emerges from hyperactivity in DCN fusiform cells, then it would be important to determine exactly what effect salicylate has on fusiform cells and other major DCN cell types, especially cartwheel cells which make glycinergic inhibitory contacts on fusiform cells. Recent studies indicate that salicylate inhibits the current mediated by glycine receptors containing alpha1-subunits (Lu et al., 2009). These results suggest that salicylate might suppress glycinergic inhibitory inputs to fusiform cells thereby increasing the firing rate of fusiform cells and suppressing IPSC. To address this issue, we used the whole-cell patch clamp technique to record from fusiform cells and cartwheel cells while perfusing salicylate onto a brain slice preparation of the rat cochlear nucleus.

2. Methods

2.1. Slice preparation

SASCO Sprague Dawley rats (aged P13–P20) were anesthetized with isoflurane and decapitated. The brainstem containing the cochlear nucleus with adjacent structures and cerebellum was cut and then glued to a cutting platform. Pseudosagittal slices (200 mm) were cut into pre-warmed (34 °C) high glucose artificial cerebral spinal fluid (HG-ACSF) containing (in mM; chemicals purchased from Sigma): 75 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 0.1 $MgCl_2$, 100 glucose, 1.36 $CaCl_2$, 4 Na L-lactate, 2 Na-pyruvate, 0.4 Na L-ascorbate, bubbled with 95% O_2 and 5% CO_2. Slices were incubated in HG-ACSF solution for at least 40 min, bubbled with 95% O_2 and 5% CO_2. Afterwards the temperature of the solution was gradually decreased to room temperature (~25 °C). Slices were transferred into a recording chamber with a continuous flow of fresh ACSF solution containing (in mM): 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 25 glucose, 1.36 $CaCl_2$ and bubbled with 95% O_2 and 5% CO_2. The recording chamber was placed under a differential interference contrast microscope (BX51WI, Olympus). Fusiform cells and cartwheel cells were identified by location within the DCN, soma shape and size, and physiological criteria. Fusiform cells were identified from their location within the fusiform layer, where they are the only large cell type, apart from cartwheel cells. Fusiform cells typically have a large, spindle-shaped soma located in the deeper part of the fusiform cell layer; their apical and basal processes project towards the molecular layer and deep layer, respectively. In contrast, the cell bodies of cartwheel cells are round, generally smaller than fusiform cells, and lie close to the surface of fusiform cell layer (Golding and Oertel, 1997). Physiologically, cartwheel cells fire unique 'complex spikes' that reliably distinguish them from fusiform cells, which fire simple, uniform spikes (Golding and Oertel, 1997; Manis et al., 1994; Zhang and Oertel, 1993).

2.2. Salicylate treatment

Our standard dose of salicylate was 1.4 mM delivered for 5 min. This concentration was selected because it matches the level of salicylate found in cerebrospinal fluid of animals injected with 460 mg/kg (i.p.) of sodium salicylate (Jastreboff et al., 1986b); previous studies have shown that salicylate doses of 150 mg/kg (i.p.) or higher reliably produce behavioral evidence of tinnitus (Jastreboff et al., 1997; Lobarinas et al., 2006). In some experiment, 1.4 mM salicylate was applied for only 3 min to evaluate the extent of recovery after shorter duration treatments. In a few studies, 5 mM salicylate was applied for 5 min to assess the effects of a higher dose.

2.3. Electrophysiology

Electrodes were pulled from glass capillary tubes (Drummond Scientific, O.D. 0.0565 inches) on a micropipette puller (Sutter, PC-84), fire polished, and wrapped with Parafilm near the tip to minimize pipette capacitance. Electrodes were filled with a potassium gluconate based internal solution containing (in mM): K-gluconate 122, NaCl 9, $MgCl_2$ 2, EGTA 0.5, HEPES 9, Tris-creatine PO_4 14, MgATP 4, Na-GTP 0.3. Whole-cell current-clamp recordings were made with an Axopatch 200B amplifier (Axon Instruments). Signals were digitized by a 16-bit data acquisition system (DIGIDATA 1320A, AXON), low-pass filtered at 5 kHz and sampled at 10 kHz. Spontaneous spikes were recorded in current clamp without applying any current. Depolarization-evoked spikes were recorded in current clamp during injection of positive current (126 pA).

Each recording session lasted 15 min and consisted of 15 one-minute epochs (Fig. 1). Negative current (−126 pA) was injected through the electrode to keep the cells hyperpolarized except during the acquisition of spontaneous activity (6 s, 0 pA) and depolarization-evoked activity (6 s, +126 pA). Input resistances were monitored through the experiment from the response to a hyperpolarization step between spontaneous and evoked recordings (Fig. 1). Only cells with stable input resistance were

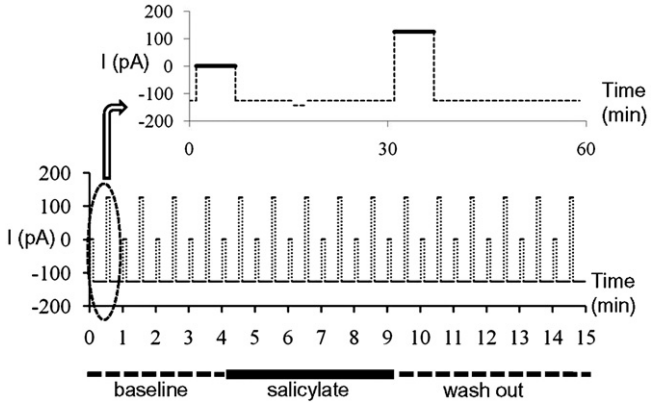

Fig. 1. Protocol for current-clamp recording. During current-clamp recording, cell injected with −126 pA except during acquisition of spontaneous and evoked spike rate when the current was 0 and +126 pA. A hyperpolarization current step was performed between two recording periods to monitor the input resistance. Salicylate was applied for 5 min starting at minute 5, followed by a washout period.