Ouabain Application to the Round Window of the Gerbil Cochlea: A Model of Auditory Neuropathy and Apoptosis

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ABSTRACT

The physiological and morphological changes resulting from acute and chronic infusion of ouabain onto the intact round-window (RW) membrane were examined in the gerbil cochlea. Osmotic pumps fitted with cannulas allowed chronic (0.5–8 days) infusions of ouabain. Acute and short-term applications of ouabain (1–24 h) induced an increase in auditory-nerve compound action potential (CAP) thresholds at high frequencies with lower frequencies unaffected. The resulting threshold shifts were basically all (no response) or none (normal thresholds), with a sharp demarcation between high and low frequencies. Survival times of 2 days or greater after ouabain exposure resulted in complete auditory neuropathy with no CAP response present at any frequency. Distortion product otoacoustic emissions (DPOAEs) and the endocochlear potential (EP) were largely unaffected by the ouabain indicating normal function of the outer hair cells (OHC) and stria vascularis. One to 3 days after short-term applications, apoptosis was evident among the spiral ganglion neurons assessed both morphologically and with TdT-mediated dUTP-digoxigenin nick end labeling (TUNEL). With 4–8 day survival times, most spiral ganglion cells were absent; however, a few cell bodies remained intact in many ganglia profiles. These surviving neurons had many of the characteristics of type II afferents. Our working hypothesis is that the ouabain induces a spreading depression among the type I ganglion cells by blocking the Na⁺,K⁺-ATPase pump. Because of the constant spike activity of these cells, the ouabain rapidly alters potassium concentrations within ([K⁺]i) and external to ([K⁺]o) the ganglion cells, thereby initiating an apoptotic cascade.

Keywords: apoptosis, auditory nerve, endocochlear potential, gerbil hearing, osmotic pump, ouabain spreading depression

INTRODUCTION

Ouabain is a well-known cardiac glycoside that specifically binds to Na⁺,K⁺-ATPase and blocks its activity. The Na⁺,K⁺-ATPase pump plays a fundamental role in cellular ion homeostasis (Alberts et al. 1983). The enzyme is a heterodimer composed of one α and one β subunit, with the α subunit being the site of attachment for ([K⁺]o) and ouabain (Lingrel and Kuntzweiler 1994; Lingrel et al. 1998). Ouabain has been used as a tool in many previous investigations of cochlear ion homeostasis (Konishi and Mendelsohn 1970; Konishi and Salt 1980; Marcus et al. 1981b), cochlear potentials (Kuijpers et al. 1967; Konishi and Mendelson 1970; Kuijpers and Bonting 1970; Sellick and Johnstone 1974; Bosher 1980), and function of the stria vascularis (Marcus et al. 1978, 1981b; Wagemann et al. 1995). It has been shown in most species to be quite effective in blocking the transport of K⁺ into...

This study evolved from our efforts to understand the functional consequences of impeding the activity of the stria vascularis, a structure which has been implicated as a primary site of age-related pathology leading to hearing loss in quiet-reared gerbils (Schulte and Schmiedt 1992; Gratton et al. 1995; Schmiedt 1996). One aspect of our studies is the development of a model of age-related hearing loss in a young animal. Furosemide has been used successfully as an agent to block the generators of the EP when applied chronically to the round window (SW) via an osmotic pump and cannula (Schmiedt 1997; Schmiedt et al. 2000). It was thus expected that ouabain similarly applied to the RW might be even more effective than furosemide in inducing long-term injury of the stria. Given the previous studies in other species, our original hypothesis was that ouabain would target the marginal cells and possibly some of the fibrocytes located within the lateral wall which stain positively for Na⁺,K⁺-AT-Pase (Schulte and Adams 1989; McGuirt and Schulte 1994; Gratton et al. 1995, 1997).

Surprisingly, the results in the gerbil revealed that the spiral ligament and stria vascularis were not permanently affected by the acute or chronic application of ouabain to the RW. Indeed, the EP remained relatively stable in these ears, as did outer hair cell (OHC) function as monitored with DPOAEs. Instead, ouabain treatment promoted a partial to complete and permanent loss of auditory-nerve function. Further investigation at the light microscopic (LM) level revealed that exposure to ouabain selectively destroyed most of the spiral ganglion neurons, leaving only a few remaining. Thus, the application of ouabain to the RW of the gerbil cochlea produces a model of auditory-nerve neuropathy, while leaving the spiral and hair cell systems relatively intact.

**METHODS**

**Animals**

Thirty-seven Mongolian gerbils (Meriones unguiculatus) of both genders and with healthy external ears were used in these experiments. Ages ranged from 4 to 7 months. Twenty-one animals were implanted with pumps with cannulas leading to the right ear; 16 animals were used in the acute experiments. Left ears were always used as controls. Animals were born and reared in an acoustically controlled colony where median sound pressure levels (SPL) were seldom greater than 40 dBA. The animal facilities have full AAALAC accreditation, and all experimental procedures were approved by the local IACUC and met NIH guidelines for animal care.

**Surgical procedures**

Surgery and the procedures for the recording of the compound action potential (CAP) response and EP have been described previously (Schmiedt and Zwislocki 1977; Schmiedt 1996; Schmiedt et al. 1996). Briefly, the animal was anesthetized with sodium pentobarbital (50 mg/kg) and fitted to a head holder located in a sound- and vibration-isolated booth. The booth was heated to maintain the cochlea at or near body temperature. Supplemental doses of anesthesia were given as needed. The core temperature of the animal was controlled by a closed-loop DC heating pad. The pinna and surrounding glands were removed and the bulla opened widely.

Acute applications of ouabain to the RW used about 100 μL of 1 mM ouabain (diluted with normal saline) to fill the RW niche. CAP thresholds were monitored during this procedure with a Ag–AgCl wire electrode resting on the surface of the bony niche. When a recovery period was desired, the RW niche was accessed using sterile procedures via a lateral incision ventral to the pinna. The ouabain was washed off after 1–2 h, depending on the acute CAP threshold shift desired. The bulla was then cemented closed, the incision sutured, and the animal allowed to recover for between 12 h and 1 week. To minimize cochlear trauma, no measures of EP were made during the actual application of ouabain to the RW for acute applications associated with a recovery period. There were no recovery periods in those acute experiments where EP was monitored continuously during ouabain application (see Figs. 4 and 5).

The survival surgery for pump implantation was done under sterile conditions. Antibiotics were not used, and none of the animals showed signs of any past or ongoing infection. Gerbils were anesthetized with sodium pentobarbital (50 mg/kg) and were given atropine (0.2 mg/kg) to reduce secretions. Alzet® mini-osmotic pumps (model 2004, Direx, Cupertino, CA) were used in all the experiments. These pumps have a fill volume of about 200 μL and a mean pumping rate of 0.25 μL/h at 37°C. Under these conditions they will last about 28 days. Cannulas were made up of surgical-grade silicon tubing. Pumps and cannulas were filled aseptically and allowed to equilibrate at 37°C for 48 h before implantation. Pumps were filled with 1 mM ouabain (diluted with sterile normal saline) and were left implanted for 12 and 24 h (short-term), or 2, 4, and 8 days (chronic). In some gerbils, terminal physiological measures were done with the pump in place; in others, the pump was removed and the animal allowed to recover for 1–4