

Furosemide alters nonlinear capacitance in isolated outer hair cells

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Abstract

The outer hair cell (OHC) from the organ of Corti plays a crucial role in hearing through its unique voltage-dependent mechanical responses. Furosemide, one of the loop diuretics, disrupts normal cochlear function. Here we report on direct effects of furosemide on OHC motility-related, voltage-dependent capacitance using the whole-cell patch-clamp technique. Extracellularly applied furosemide reversibly shifted the voltage at peak capacitance (V_{pkC_m}) to positive levels. The shift, whose maximum approached 90 mV, evidenced a Hill coefficient of 1.5 and $K_{1/2}$ of 10 mM. Changes in the magnitude of nonlinear capacitance were not fully reversible. While it is clear that the overwhelming effect of furosemide on hearing results via its effects on the endolymphatic potential, the present results indicate that furosemide directly alters OHC motility and may, in part, contribute to sensory dysfunction. © 2001 Published by Elsevier Science B.V.

Key words: Outer hair cell; Motility; Nonlinear capacitance; Basilar membrane tuning

1. Introduction

The outer hair cell (OHC) from the organ of Corti is believed to provide feedback into the basilar membrane through its unique voltage-dependent mechanical responses (Ashmore, 1987; Brownell et al., 1985; Santos-Sacchi and Dilger, 1988). The feedback sharpens the passive mechanical vibration of the cochlear partition. The OHC has a nonlinear gating charge movement or, equivalently, a voltage-dependent capacitance which presents characteristics similar to those of OHC motility, indicating that membrane-bound voltage sensor-motor elements control OHC length (Ashmore, 1990; Dallos et al., 1993; Santos-Sacchi, 1991, 1993).

Furosemide, one of the loop diuretics, is ototoxic. Systemic or intracochlear administration of furosemide induces changes in receptor potentials (Forge and Brown, 1982), auditory -nerve responses (Evans and Klinke, 1982) and basilar-membrane vibration (Ruggero and Rich, 1991). Furosemide is thought to exert its effects on these stimulus-related responses via inter-

ference with stria vascularis function (Brown and McElwee, 1972; Kusakari et al., 1978; Wangemann, 1995), namely, through a reduction of the positive endolymphatic potential (EP). Ruggero and Rich (1991) suggested that a decrease in basilar membrane vibration at near-characteristic frequencies (CF) results from an indirect action of furosemide on OHCs via a reduction of receptor potentials, the presumed driving force for OHC motility. They concluded that the sensitivity and frequency selectivity of basilar membrane responses are due to the mechanical activity of OHCs. In order to examine whether furosemide may interfere with OHC motility more directly, we studied the effects of furosemide on OHC motility-related nonlinear capacitance using the whole-cell patch-clamp technique.

2. Methods

OHCs were freshly isolated from the organ of Corti of the guinea-pig cochlea, following death by anesthetic overdose with Nembutal. The procedures were in accordance with Yale University's Animal Use And Care Committee guidelines. OHCs were whole-cell voltage clamped using patch pipets with initial resistances of

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2–3 M Ω . Series resistance ranged from 3 to 5 M Ω . The patch-pipette solution contained (in mM): 140 CsCl, 2 MgCl₂, 10 EGTA and 10 HEPES, with pH 7.2 and 300 mOsm. Ionic blocking solutions were used to remove voltage-dependent ionic conductances so that capacitive currents could be analyzed in isolation. The external solution contained (in mM): 100 NaCl, 20 TEA, 20 CsCl, 2 CoCl₂, 1.52 MgCl₂, 10 HEPES and 5 dextrose, pH 7.2, 300 mOsm. Furosemide was tested in concentrations ranging from 0.5 to 30 mM, with NaCl adjusted to maintain osmolarity. Drugs were applied via cell-chamber perfusion, or in some cases via Y tube. Voltages were corrected for the effects of residual series resistance.

Whole-cell membrane capacitance was measured with two techniques, transient and AC. The former entailed the delivery of a stair-step voltage stimulus. Transient currents were integrated to obtain cell capacitance (C_m) measures as previously described (Huang and Santos-Sacchi, 1993). The latter technique utilized a continuous high-resolution (2.56 ms sampling) two-sine voltage stimulus protocol (10 mV peak at both 390.6 and 781.2 Hz), with subsequent fast Fourier transform-based admittance analysis (Santos-Sacchi et al., 1998). These high-frequency sinusoids were superimposed on voltage-ramp stimuli. Capacitance data were fit to the first derivative of a two-state Boltzmann function (Santos-Sacchi, 1991),

$$C_m = Q_{\max} \frac{ze}{kT} \frac{b}{(1+b)^2} + C_{\text{lin}} \quad (1)$$

$$b = \exp\left(\frac{-ze(V_m - V_{\text{pk}C_m})}{kT}\right)$$

where Q_{\max} is the maximum nonlinear charge moved, $V_{\text{pk}C_m}$ is voltage at peak capacitance or half-maximal nonlinear charge transfer, V_m is membrane potential, C_{lin} is linear capacitance, z is valence, e is electron charge, k is Boltzmann's constant, and T is absolute temperature. $V_{\text{pk}C_m}$ was tracked in real time with 2 mV resolution by monitoring the reversal of gating current polarity as fully described elsewhere (Kakehata and Santos-Sacchi, 1995).

3. Results

The inset of Fig. 1 illustrates the effect of furosemide on $V_{\text{pk}C_m}$ of an isolated OHC utilizing the $V_{\text{pk}C_m}$ tracking procedure. The drug (20 mM) shifted $V_{\text{pk}C_m}$ from a resting value of -65 mV to about $+20$ mV; washout reversed the effect. In order to prevent interference by potential turgor pressure changes (Kakehata and Santos-Sacchi, 1995), the cylindrical cell was collapsed with

negative pipette pressure from the outset of recording. The dose response curve for the shift in $V_{\text{pk}C_m}$ (Fig. 1) indicates a Hill coefficient of 1.5, K_m of 10.06 mM, and maximum shift of 86.9 mV. Significant shifts were seen at low concentrations, i.e. at 1 mM, the average shift was 5.96 ± 1.43 mV ($n = 18$; mean \pm S.E.M.).

Furosemide also reduced the magnitude of nonlinear capacitance in a graded manner (Fig. 2, inset). For the cell depicted in Fig. 2, $C_{m,\text{pk}}$ decreased from 36.2 pF to 35.6, 34.0, 32.3 and 30.9 pF at 0.7, 4, 15 and 20 mM, respectively. The corresponding positive shifts in $V_{\text{pk}C_m}$ were 12, 20.9, 62.4 and 75.6 mV. However, the effects of furosemide on the magnitude and voltage dependence of OHC capacitance were not necessarily correlated. Upon washout, whereas $V_{\text{pk}C_m}$ typically returned to near-initial conditions, nonlinear capacitance usually remained depressed. Since OHC capacitance and mechanical functions coincide (Kakehata and Santos-Sacchi, 1995; Wu and Santos-Sacchi, 1998), this depression corresponds to a change in the form of the OHC electromechanical function.

4. Discussion

A correspondence between OHC nonlinear capacitance and motility indicates that the lateral membrane motor and sensor are tightly coupled or one entity (Santos-Sacchi, 1991; Kakehata and Santos-Sacchi, 1995); the motor is likely the recently identified protein prestin (Zheng et al., 2000; Santos-Sacchi et al., 2001). We demonstrate here that furosemide directly alters the OHC's peak capacitance and $V_{\text{pk}C_m}$, demonstrating that the drug directly affects the mechanical activity of the OHC. The magnitude of its effect is one of the largest of any drug measured to date. Interestingly, the peak capacitance does not recover with the same time course as $V_{\text{pk}C_m}$, indicating that the voltage sensitivity of the mechanical response is more sensitive to furosemide than is its operating point along the voltage axis. It should be noted that the Boltzmann model parameters that describe the nonlinear capacitance are not necessarily linked, and it is not unusual to independently affect one but not another. For example, $V_{\text{pk}C_m}$ could shift simply due to a change in membrane surface potential, while all other parameters remain constant.

The mechanism whereby furosemide affects the OHC motor is not clear. Furosemide, which is a known inhibitor of chloride transport (see Wangemann, 1995), is also reported to block Cl channels, such as γ -aminobutyric acid A (Nicoll, 1978; Pearce, 1993) and Ca²⁺-dependent Cl channels (Evans et al., 1986). Despite the existence of a potentially susceptible Cl channel in the lateral membrane (Gitter et al., 1986; Ohnishi et al., 1993), we have ruled out consequent effects on turgor

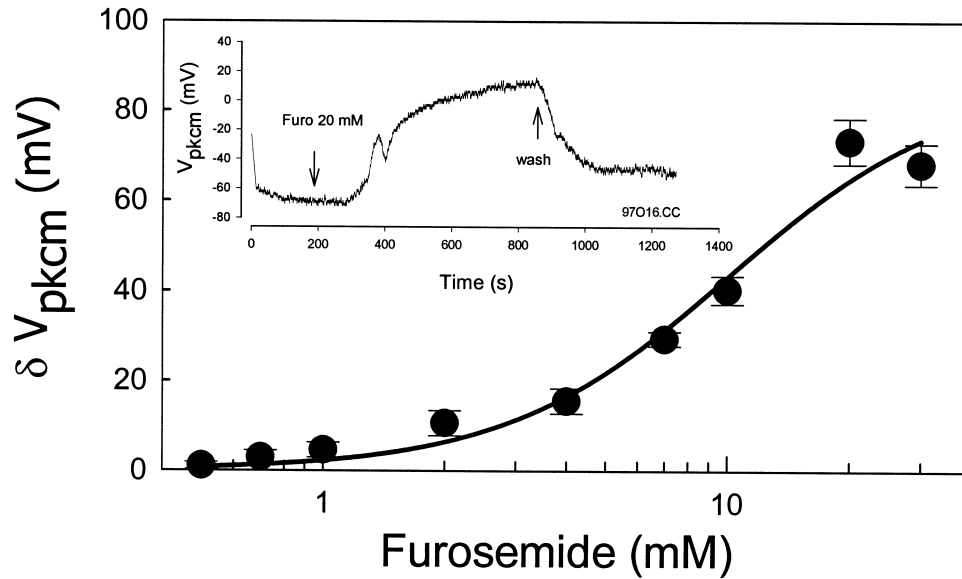


Fig. 1. Effect of furosemide on V_{pkC_m} . Inset: V_{pkC_m} as a function of time after whole-cell voltage-clamp configuration was obtained with the tracking technique. Furosemide (20 mM) was applied by bath. The delay in response is due to time required for bath solution exchange. Cell was collapsed from the outset of recording. Note reversible shift in V_{pkC_m} . The main figure shows three parameter Hill fit giving a Hill coefficient of 1.5, K_m of 10.06 mM, and maximum shift of 86.9 mV. Data points are mean \pm S.E.M., with observation number ranging from 3 to 10.

pressure by collapsing the OHCs. It may be significant that furosemide can also block sulfate transport (Wolpaw and Martin, 1986) and that the recently identified OHC motor protein, prestin, is homologous to sulfate transporters (Zheng et al., 2000).

The predominant ototoxic effects of furosemide on cochlear function are believed to result from a drop

in the EP (Evans and Klinke, 1982; Sewell, 1984; Wangemann, 1995). However, while Ruggero and Rich (1991) conclude that furosemide-induced decreases in basilar membrane vibration at near-CF are via an indirect action of furosemide on OHCs, there is some evidence that furosemide treatments can affect OHCs directly (Comis et al., 1990; Forge and Brown, 1982).

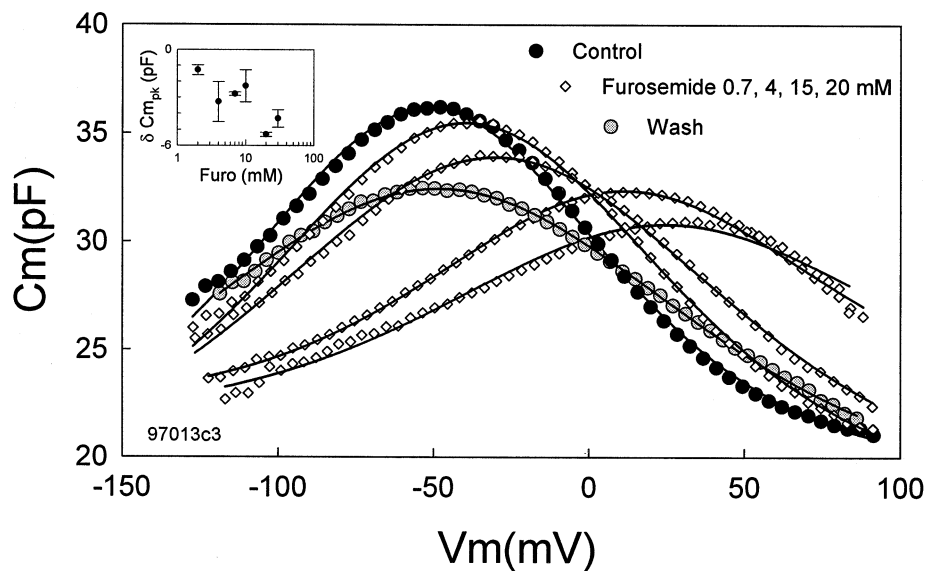


Fig. 2. Effect of furosemide on OHC $C-V$ function. Inset: Change in peak capacitance as a function of furosemide concentration (mean \pm S.E.M.). The main plot shows, for an individual OHC, the effects of 0.7, 4, 15, and 20 mM furosemide (diamonds, successively decreasing peaks) on the cell's $C-V$ function. Control (filled circle) and wash conditions (gray circle) are shown as well. Fits (solid lines) are to Eq. 1. Fitted parameters: control (V_{pkC_m} , z , Q_{max} , C_{lin} , respectively): -51.51 , 0.67 , 2.57 , 19.35), 0.7 mM (-39.47 , 0.60 , 2.95 , 18.19), 4 mM (-30.11 , 0.52 , 3.06 , 18.13), 15 mM (11.36 , 0.61 , 1.69 , 22.19), 20 mM (24.67 , 0.53 , 1.76 , 21.51) and wash (-48.44 , 0.48 , 3.03 , 18.09).

Indeed, it has recently been demonstrated that otoacoustic emissions, which are thought to reflect OHC mechanical activity, are capable of recovering from the effects of furosemide treatment prior to the recovery of the EP (Mills et al., 1993). These data indicate that EP alterations may not solely underlie furosemide's effects on cochlear function.

Ostensibly, the strongest argument that intravenous furosemide works solely on the stria vascularis, without direct effects on OHC function, is the rapidity of its adverse effects on eighth-nerve function; perilymphatic perfusion apparently requires longer times and high concentrations, and Evans and Klinke (1982) and Sewell (1984) are frequently quoted in support of this argument. However, Evans and Klinke report only data from one experimental attempt at perilymphatic perfusion, and Sewell cites his unpublished observations. It is invariable that during surgical preparation for perilymphatic perfusion cochlear function is depressed by 20 dB or greater (Sewell, personal communication). Thus, during perilymphatic perfusion of furosemide, if the effects were smaller than 20 dB they would not have been observed. Since we show that the effect of furosemide is immediate on isolated OHCs, we would expect a rapid effect via perilymphatic perfusion of the mid-micromolar concentrations instilled by Evans and Klinke. We conclude that effects were masked by a compromised cochlear.

It is expected that either a shift in the OHC membrane potential or the voltage dependence of the cell's mechanical response may modify the OHC's feedback into the basilar membrane, since the gain of the OHC mechanical response will be modified (Takehata and Santos-Sacchi, 1996). Small changes in feedback can have pronounced effects. For example, the major efferent neurotransmitter, acetylcholine, maximally hyperpolarizes the OHC membrane potential a few millivolts (Housley and Ashmore, 1991). Such an effect may underlie the observation by Dolan et al. (1997) that stimulation of the OHC efferent system is capable of modifying basilar membrane mechanics in vivo. How effective might furosemide be in altering OHC activity in the living animal, given its large K_m of 10 mM? Mid-micromolar concentrations of furosemide can shift V_{pkC_m} by about 1 mV. By comparison, salicylate, another ototoxic agent which has measurable consequences at perilymphatic concentrations of 400 μ M (Jastreboff et al., 1986), shifts V_{pkC_m} less than the same concentration of furosemide (Takehata and Santos-Sacchi, 1995). Following intravenous administrations of ototoxic furosemide levels, perilymphatic concentrations are cleared more slowly than in serum, but reach only low micromolar concentrations ($\sim 15 \mu$ M) (Rybak et al., 1979). Interestingly, immature animals accumulate higher perilymphatic levels (Mills et al., 1997).

While the effects of such low furosemide concentrations on cochlear function in the absence of EP effects has yet to be determined, it can be reasonably concluded that a compromised EP, as occurs with intravascular administration, will have a predominant effect on cochlear function.

Acknowledgements

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