Fast, But Not Slow, Effects of Olivocochlear Activation Are Resistant to Apamin

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Olivocochlear (OC) effenter suppression of auditory-nerve responses comprises a fast effect lasting tens of milliseconds and a slow effect building and decaying over tens of seconds. Both fast and slow effects are mediated by activation of the same alpha 9 nicotinic receptor. We have hypothesized that fast effects are generated at the OC synapse, but that slow effects reflect activation of calcium-activated potassium (KCa) channels by calcium release from the subsurface cisternae on the basolateral wall of the hair cells. We measured in vivo effects of apamin, a blocker of small-conductance (SK) KCa channels, and charybdotoxin, a blocker of large-conductance KCa channels, perfused through scala tympani, on fast and slow effects evoked by electrical stimulation of the OC bundle in anesthetized guinea pigs. Apamin selectively and reversibly reduced slow-effect amplitude without altering fast effects or baseline amplitude of the auditory-nerve response. The very high concentrations of apamin needed to block efferent effects contrasts with the sensitivity of isolated hair cells to apamin’s block of acetylcholine’s effects. The results suggest that in vivo fast OC effects are dominated by a conductance that is not apamin sensitive.

INTRODUCTION

In the mammalian cochlea, outer hair cells (OHCs) and inner hair cells (IHCs) act in concert to transduce auditory signals. OHCs amplify sound-evoked motions of the basilar membrane (reviewed by Dallos 1996), while IHCs respond by releasing a neurotransmitter to excite the auditory nerve fibers contacting them. This combined process produces the high sensitivity and sharp tuning seen throughout the ascending auditory pathways. The brain can suppress cochlear sensitivity via activity in olivocochlear (OC) effenter nerve fibers that synapse on OHCs. These OC effects are mediated by nicotinic alpha 9 cholinergic receptors (Elgoyhen et al. 1994; Vetter et al. 1999) and comprise two components: a fast effect lasting tens of milliseconds and a slow effect, building up and decaying over tens of seconds. Fast effects elevate cochlear thresholds (Galambos 1956); slow effects further elevate threshold (Sridhar et al. 1995) and may have an additional role in protecting the ear from acoustic trauma (Reiter and Liberman 1995).

While both fast and slow effects are initiated by the alpha 9 nicotinic receptor, their different time courses suggest different intracellular mechanisms. Most studies of effenter stimulation (Art et al. 1984) or of application of acetylcholine (ACh) (Blanchet et al. 1996; Evans 1996; Fuchs and Murrow 1992; Housley and Ashmore 1991; Nenov et al. 1996; Yuhas and Fuchs 1999) on hair cells in vitro suggest that calcium entry through the alpha 9 receptor directly activates KCa channels to hyperpolarize the hair cell. We have hypothesized (Sridhar et al. 1997) that the slow effect is generated when focal calcium entry through the alpha 9 receptor at the effenter synapse triggers propagation of calcium-induced calcium release from the synaptic and subsurface cisternae to activate KCa channels on the basolateral wall of the OHC, i.e., distant from those producing the fast effect. One possibility is that fast and slow effects are mediated via different KCa channels. ACh effects on isolated hair cells can be blocked by apamin, an antagonist of the small conductance KCa channel, but not by charybdotoxin, a blocker of large conductance KCa channels (Doi and Ohmori 1993; Nenov et al. 1996; Yamamoto et al. 1997; Yoshida et al. 1994; Yuhas and Fuchs 1999). As a means of relating in vivo cochlear work to that in isolated hair cells, the present study attempts to pharmacologically separate fast and slow OC effects with cochlear perfusion of apamin or charybdotoxin.

METHODS

We perfused scala tympani of guinea pigs with an artificial perilymph solution, while recording the effects of electrical stimulation of OC nerve fibers on the compound action potential (CAP). Albino guinea pigs of either sex, weighing 350–600 g, were anesthetized with urethan (1.5 g/kg ip), droperidol (2 ml/kg im), and fentanyl (2 ml/kg im). The animals received boosters of urethan (1/3 the original dose) after 6–8 h, and boosters of droperidol and fentanyl (1/3 the original dose) every 2 h. Animals were tracheostomized and connected to an artificial respirator. The temperature within the experimental chamber was maintained at 32–33°C. A heating blanket was used to maintain the rectal temperature of the animal between 37 and 39°C. The pinnae were removed, and the cochlea was exposed by a dorsolateral approach. Acoustic stimuli were produced by a 1-in.
condenser microphone driven as a sound source and were measured on-line by a ¼-in. microphone and probe tube assembly housed in a brass coupler, which sealed tightly around the cartilaginous portion of the external ear.

To measure the CAP and cochlear microphonics, gross electric potentials that represent the summed activity of the auditory nerve fibers and the OHCs, respectively, a silver-wire electrode was placed near the round window, and an indifferent electrode was connected to the tongue or placed in the neck muscles. Responses to acoustic stimuli (see METHODS) were amplified ×10,000 by an AC-coupled amplifier (pass-band 100–10,000 Hz). The resulting signal was digitized with 30-μs sampling intervals via a 12-bit A/D converter (National Instruments A2000), and the digital waveforms were averaged on-line using custom software in LabView (National Instruments) on a Macintosh computer.

A posterior craniotomy was performed, and a portion of the cerebellum was aspirated to expose the floor of the IVth ventricle. The olivocochlear bundle (OCB) was stimulated electrically with electrodes placed on the floor of the IVth ventricle at the midline, where the OCB runs close to the surface of the brain stem (White and Warr 1983). The stimulator consisted of a rake of six fine silver wires placed at 0.5-mm intervals. After placement of the rake along the brain stem midline, different pairs of electrodes were assayed to find the optimum pair for eliciting OC activity. Shocks were always monophasic pulses of 150-μs duration. Shock levels were typically set 5–10 dB above threshold for facial twitches in the absence of the paralytic. Since electrical stimulation of the OCB can cause muscle twitches, muscle paralysis was induced with D-tubocurarine (1.25 mg/kg im) and maintained with boosters as necessary. OCB-induced changes in cochlear microphonic and CAP were determined from the digitized waveform.

Our electrophysiological assay allowed simultaneous quantification of fast and slow effects of OC stimulation. It presents brief acoustic stimuli (see METHODS) were amplified ×10,000 by an AC-coupled amplifier (pass-band 100–10,000 Hz). The resulting signal was digitized with 30-μs sampling intervals via a 12-bit A/D converter (National Instruments A2000), and the digital waveforms were averaged on-line using custom software in LabView (National Instruments) on a Macintosh computer.

RESULTS

Apamin, a peptide of 2,200 MW, was perfused through the scala tympani at concentrations from 10 to 300 μM while we electrically stimulated OC efferents and recorded CAP responses. Given its relatively large size, and the presence of peptidases in the organ of Corti, it is likely that apamin concentration in our perfuse is significantly higher (10- to 100-fold) than that achieved at the OC/OHC synapse (see DISCUSSION).

The effects of apamin were dose dependent and selective. The most reproducible results were seen at 100 μM, where apamin reversibly reduced slow-effect amplitude without significantly altering either the baseline CAP or the fast effect. Data from one...
experiment are shown in Fig. 2, in which the slow effect was reduced by more than 50% by the end of the 20-min perfusion with apamin. CAP and fast OC effect amplitudes were unchanged. The reproducibility of these effects of 100 μM apamin on CAP amplitude, fast OC effects, and slow OC effects are demonstrated in Fig. 3, where data from four different experiments are averaged. In these mean data, 100 μM apamin reversibly reduced the magnitude of the slow OC effect to less than half its original amplitude, with a time course reflecting the presence of apamin in the perilymph. At 10 μM, apamin had no discernable effect on the baseline CAP or on OC effects (fast or slow). At 20–30 μM, no consistent effect was easily observed in the raw data, although quantification of the average changes during apamin perfusion showed a small (24%) suppression of slow OC effect (Fig. 4). CAP was unchanged, and fast effect was suppressed by <10%.

The effects of charybdotoxin were complex. At concentrations of 10 nM and lower, it produced no consistent effect. At concentrations of 30–100 nM, charybdotoxin had effects with two different time courses. An initial effect was an enhancement of the slow effect (shown in Fig. 5 and noted in 2 other cases at 30 nM). This initial effect occurred with the same time course as that seen with other drugs acting on the outer hair cell (Sridhar et al. 1995, 1997; Yoshida et al. 1999). The enhancement of slow effect was often associated with a moderate reduction in CAP. A later effect was a large reduction in baseline CAP amplitude, as well as the magnitude of fast and slow OC effects (Fig. 5). The time course of reduction in CAP and recovery was significantly longer than for apamin and other OHC efferent blockers we have tested, suggesting a different site of action for this late effect.

DISCUSSION

The numerous reports that apamin blocks cholinergically induced potassium currents in isolated hair cells leave little
doubt that this current in vitro is mediated by apamin-sensitive $K_{Ca}$ channels (Doi and Ohmori 1993; Nenov et al. 1996; Yamamoto et al. 1997; Yoshida et al. 1994; Yuhas and Fuchs 1999). Our experiments address the in vivo role of these channels in mediating cholinergic OC effects in the cochlea. We found that neither the fast nor the slow effects were very sensitive to apamin, although slow effects were partially blocked by apamin. Our findings were unexpected considering that the effects of acetylcholine on isolated hair cells are highly sensitive to apamin. The high concentrations of apamin required to block slow effects, and the complete resistance of fast effects to apamin blockade, raise questions in comparing the in vivo and in vitro results.

Yamamoto et al. (1997) and Yuhas and Fuchs (1999) partially blocked the ACh response in isolated hair cells at nanomolar concentrations of apamin (although, in the latter study, the dose-response curve suggested 2 components, with a significant portion unblocked until $\mu$M concentrations were delivered). On the other hand, we did not find any significant blockade of OC effects until concentrations were between 20 and 100 $\mu$M, suggesting potency differences in vivo of 100- to 100,000-fold compared with the least apamin-sensitive and most apamin-sensitive in vitro responses, respectively. Some of this difference in potency may be due to fundamental differences in drug access for in vitro versus in vivo experiments. With isolated hair cells, drug delivery is relatively direct, and bath concentration should equal concentration at the hair cell. For in vivo perfusions, on the other hand, drugs must diffuse through a number of intercellular spaces to reach the hair cells from scala tympani. Our previous in vivo study of a $\alpha_9$ nicotinic blocking agents (Sridhar et al. 1995) found EC$_{50}$s 10–100 times higher than those for the same drugs in isolated hair cell experiments (Dallos et al. 1997; Doi and Ohmori 1993; Elgoyhen et al. 1994; Fuchs and Murrow 1992; Housley and Ashmore 1991; Tucker and Fettiplace 1996). Despite the diffusional barriers, peptides perfused through scala tympani can reach the organ of Corti. For example, we show effects of perfused charybdotoxin at 30 nM (Fig. 5), and Kujaee et al. (1994) saw in vivo effects of bungarotoxin (a peptide acting at the ACh receptor) at submicromolar concentrations.

Even if diffusional barriers can account for up to a 100-fold concentration difference, this does not fully explain the observed discrepancy. It is unlikely that high concentrations required in the present study arise from degradation of the apamin. For each experiment, we used a new batch of apamin, placed into solution just prior to cochlear perfusion. Furthermore, Bobbin and LeBlanc (1999) were also unable to block in vivo fast OC effects with perfused apamin at concentrations up to 1 mM (at which point enough apamin apparently leaked into the cerebrospinal fluid to produce convulsions). Yet, Bobbin’s laboratory has blocked 95% of ACh effects on isolated hair cells with 1 $\mu$M apamin (Nenov et al. 1996), suggesting the potency differences are not laboratory dependent.

Our results clearly show that apamin does reach the hair cells in vivo, given that selective blockade of the slow effect was the first change seen as apamin concentration was increased (Figs. 3 and 4). The observed blockade is consistent with an action on OHCs. Indeed, it is difficult to imagine another site of action. Slow and fast effects are both generated by the OC/OHC synapse, as evidenced by their presence in microphonic potentials (generated by OHCs) as well as the neural responses measured here and by their simultaneous blockade by $\alpha_9$ cholinergic antagonists (Sridhar et al. 1995).

The two actions of charybdotoxin with different time courses suggest two sites of action with different access paths from the scala tympani. The early action, to enhance the slow effect, is consistent with an action on OHCs; that action occurred with the same time course as the action of many other drugs we have used that affect the OHC. If either fast or slow effect was produced by large-conductance potassium (BK) channels, then one would have expected charybdotoxin to block the effect. The enhancement of slow effect with charybdotoxin might argue against a direct involvement of BK channels in OC effects. However, because of the drastic changes in CAP with charybdotoxin, we did not use higher concentrations and thus cannot rule out some involvement of BK channels in efferent effects.

Another possibility, based on differential sensitivity of slow OC effects to apamin, is that acetylcholine application to isolated hair cells may produce effects more similar to in vivo slow OC effects than to fast OC effects. Murugasu and Russell (1996) reached a similar conclusion in their work on the effects of ACh on basilar membrane movement. Although effects of cholinergic agonists on isolated hair cells can be rapid (Chan and Evans 1998), the sluggishness of the in vivo slow effect
may arise from the slow buildup of a calcium “spark” propagating through the subsurface cisternae (Sridhar et al. 1997). In isolated hair cells, activation of large numbers of ACh receptors by pharmacological application of cholinergic agents may activate apamin-sensitive KCa channels without the need for a propagating calcium spark or may more rapidly generate a calcium spark. Arguing against this hypothesis is the recent finding of Oliver et al. (2000), who infer the involvement of SK2 channels in rapid efferent effects after evoking ACh release in vitro from efferent nerve terminals attached to isolated hair cell; however, Oliver et al. (2000) did not use apamin.

One can also apply in vivo findings to the study of isolated hair cells: we have characterized a number of physiological and pharmacological differences between fast and slow effects that should have correlates in isolated hair cell responses. Slow effects desensitize while fast effects do not. Slow effects are enhanced, and desensitization is blocked, by cyclopiazonic acid and thapsigargin, while fast effects are unaffected by these agents.

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REFERENCES


