Regulation of Auditory Responses in the Central Nucleus of the Inferior Colliculus by Tetraethylammonium-Sensitive Potassium Channels

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Zhang, Huiming, Shu Hui Wu, and Jack B. Kelly. Regulation of auditory responses in the central nucleus of the inferior colliculus by tetraethylammonium-sensitive potassium channels. J Neurophysiol 91: 2194–2204, 2004; 10.1152/jn.00730.2003. The role of potassium channels in regulating spontaneous firing and sound-evoked responses in the central nucleus of the inferior colliculus was studied by recording single-unit activity before and during iontophoretic application of a nonspecific potassium channel blocker, tetraethylammonium (TEA). Tone bursts and sinusoidal amplitude-modulated tones were used to evoke auditory responses. Our results show that release of TEA increased the width of spikes for all neurons tested. There was an increase in spontaneous firing for most of the neurons. There was also an increase in responses to tone bursts for most of the neurons, although in some cases there was a reduction in the evoked responses. TEA also increased the firing rate in responses to sinusoidal amplitude-modulated sounds in the majority of the neurons tested. For some neurons, the change in firing reduced the selectivity of responses for particular rates of modulation. There was also a reduction in the synchrony of action potentials to the modulation envelope in many cells. Our results show that potassium channels are important for regulating the strength of sound-evoked responses and the level of spontaneous activity, and determining the temporal properties of responses to amplitude-modulated sounds.

INTRODUCTION

A neuron’s physiological responses are determined primarily by its intrinsic membrane properties and the neural circuits with which it is associated. Neural circuits establish the number, nature, and location of synapses, as well as the strength and timing of synaptic inputs. Intrinsic membrane properties establish the way in which a neuron integrates and modifies incoming synaptic signals. The number and characteristics of ion channels are important determinants of intrinsic membrane properties.

Potassium channels play a critical role in generating neural responses and governing neuronal excitability (Hille 2001). Intracellular recordings from neurons in brain slice preparations of the auditory brain stem have shown that potassium channels contribute to neuronal activity in various ways (Bal and Oertel 2001; Brew and Forsythe 1995; Fu et al. 1996; Rathouz and Trussell 1998; Sivaramakrishnan and Oliver 2001; Svirskis et al. 2002; Wang et al. 1998). For instance, bushy cells in the ventral cochlear nucleus possess low-threshold old potassium channels that serve to reduce the membrane time constant and shorten the time course of synaptic events (Manis and Marx 1991). Consequently, bushy cells are able to preserve precisely the temporal structure of inputs from auditory nerve fibers and convey it to higher auditory centers. Potassium channels also help to maintain resting membrane potentials in auditory neurons. In the ventral cochlear nucleus some potassium channels that are sensitive to tetraethylammonium (TEA, a nonspecific potassium channel blocker) are open when cells are at or near the resting potential (Manis and Marx 1991). In the dorsal cochlear nucleus, blocking potassium channels with bath application of TEA results in a substantial depolarization of the cell membrane (Agar et al. 1996; Hirsch and Oertel 1988). A recent brain slice study of the rat’s central nucleus of the inferior colliculus (ICC) has shown that the firing pattern of a neuron in response to depolarizing and hyperpolarizing current injection is associated with specific potassium channels (Sivaramakrishnan and Oliver 2001), most of which are subject to block by TEA.

Although in vitro studies have demonstrated that potassium channels are important in generating specific firing patterns, shaping the time course of action potentials, maintaining resting potentials, and determining membrane time constants, it is not yet clear how they regulate in vivo spontaneous activity and sound-evoked responses. The present study was designed to address this issue by observing changes in neural activity during pharmacological block of potassium channels by iontophoresis of TEA at the recording site. We recorded neural responses from the ICC because this structure is important in the integration and modification of auditory information. Our previous in vivo recordings from the rat’s ICC suggested that potassium channels might be involved in the regulation of auditory responses (Zhang and Kelly 2001, 2003). In the present study, we investigated the effects of TEA on the level of spontaneous and sound-evoked activity, and the temporal characteristics of responses to tone bursts and sinusoidal amplitude-modulated (AM) sounds.

METHODS

Animal preparation

Experiments were conducted on 25 male adult Wistar albino rats (Rattus norvegicus; 250–500 g) obtained from Charles River Canada (St. Constant, Quebec). The external ears and tympanic membranes of animals were examined with an otoscope and determined to be free of infection or other abnormalities. Surgical anesthesia was induced initially by combined injection of ketamine hydrochloride [60 mg/kg, intramuscularly (im)] and xylazine hydrochloride (10 mg/kg, im). Supplemental injections of ketamine hydrochloride (20 mg/kg, im) and xylazine hydrochloride (3.3 mg/kg, im) were made as needed.
throughout the course of an experiment to maintain a state of areflexia. Although ketamine is known to be a competitive NMDA receptor antagonist, the dosages used here are not enough to substantially affect the outcome of the study (Zhang and Kelly 2001, 2003).

A midline incision was made in the scalp, the skin and muscles were retracted laterally, and a small craniotomy was made to permit insertion of a pipette assembly into the ICC for recording neural responses and releasing TEA. Small bone screws were placed in the skull and fixed to a stainless steel rod with dental acrylic. The rod was then attached to a stereotaxic instrument (Kopf Instruments) to hold the head firmly in place while leaving the external ear canals free for insertion of earphone drivers. Recordings were made with the rats inside a single-wall sound-attenuated booth (Eckel Industries).

All procedures were approved by the Carleton University Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

**Sound stimulation**

Sounds were presented monaurally to the right ear through a sealed headphone assembly (Beyerdynamic DT 48) connected to a hollow speculum that was inserted into the rat’s external meatus. To reduce acoustic cross talk, a second speculum with a headphone assembly was inserted into the left external meatus, although no sound was delivered to this ear. The acoustic waveforms were generated digitally by MALab 881 software controlled by a Macintosh G-3 computer. The sound-generation and data-acquisition system (MALab 881) was designed and produced by Steve Kaiser, Department of Neurobiology, University of California, Irvine (Kaiser Instruments).

The acoustic stimuli used in this study included noise bursts, tone bursts, and sinusoidal AM sounds. Noise bursts (100-ms duration, 10-ms rise/fall time) were used as search stimuli. Tone bursts of 100 ms with 10-ms rise/fall time were used to determine a single neuron’s characteristic frequency (CF, the frequency at which the neuron showed the lowest response threshold) and rate-level function at CF. AM tones (10-s duration, 10-ms rise/fall time) were used to study the synchronization of discharges to the modulation envelope at various rates of modulation from 0.5 to 1,000 Hz. The carrier frequency of an AM tone was set at the cell’s CF and the depth of modulation was fixed at 100%. Tone bursts were presented 20 times at a rate of 1/s to generate summed neural responses, and AM tones were presented 2 times with a rate of 1 every 12 s to generate summed responses.

The sound pressure level (SPL) for all stimuli was calibrated over a frequency range of 100–40,000 Hz using a condenser microphone (Bruel and Kjaer 4134) with the headphone assembly fitted into an enclosed chamber constructed from tygon tubing. The acoustic response of the headphone assembly was adjusted on-line to provide a constant sound pressure over the range from 500 Hz to 30 kHz.

**Recording and drug delivery**

A piggyback multibarrel glass electrode assembly (Havey and Caspary 1980) was used for extracellular recording and iontophoretic delivery of drugs. The electrode was fabricated by fixing a single-barrel recording micropipette to a 5-barrel glass pipette with cyanacrylate glue (Viachem, Montreal, Quebec) at an angle of about 20°. The tip of the single-barrel pipette was 2–3 μm in diameter, and the tip of the 5-barrel pipette was 8–10 μm. The recording electrode was filled with 3 M potassium chloride. Two barrels of the 5-barrel pipette were filled with TEA (500 mM, pH 7.0, Sigma) in a vehicle of 165 mM sodium chloride. The remaining 3 barrels were filled with 165 mM sodium chloride to maintain an electrical balance across the injection pipette tip.

The electrode assembly was driven by a Kopf model 650 micropositioner. The multibarrel pipette was connected to iontophoresis pumps of a Neurophore-BH-2 micro-iontophoresis system (Biomedical Systems). Neural activity registered by the single-barrel pipette was amplified by a Dagan EX 4-400 Quad differential amplifier and monitored audiovisually. Neural responses were digitized and sampled using an A/D converter and MALab 881 software. The occurrence time of spikes was recorded with a resolution of 1 μs, stored on computer, and processed later with standard database and graphics software.

**Recording procedure**

During a recording session, the left ICC was approached obliquely with the electrode tilted by 30° relative to the sagittal plane and stereotaxic coordinates referenced to a point 3.8–5.0 mm lateral and 0.3–0.5 mm rostral to lambda. The electrode was then lowered into the brain to a depth between 2.3 to 6.0 mm while monitoring responses to acoustic stimulation. As the electrode was lowered into the brain, a retaining current of ~10 nA was applied to the pipette barrels containing TEA to prevent leakage of the drug during the search for single-unit activity and during predrug recording or postdrug recovery phases of the experiment.

Single-unit activity was recognized by spikes of constant amplitude and waveform. A window discriminator was used to isolate spikes from background activity. We typically used a noise burst presented to the right ear to search for responsive neurons. After an auditory neuron had been identified, tone bursts of variable frequency and amplitude were used to determine the CF on the basis of spike counts and audiovisual monitoring of driven responses. Tone bursts at the cell’s CF and at various SPLs were then used to determine the rate-level function. After the neuron was characterized and responses to tone bursts were recorded, we delivered sinusoidal AM tones with the carrier frequency at the cell’s CF and the SPL typically fixed at 15 to 20 dB above the threshold at CF. The modulation frequency was systematically changed from 0.5 to 1,000 Hz.

TEA was delivered iontophotically at the recording site to determine how potassium channels contribute to single-unit responses. The currents used to release the drug were +5 to +50 nA (typically ~+20 nA). The effect of the TEA on the waveform of a spike was monitored constantly. The drug effect was also monitored by repeatedly recording spontaneous activity and sound-evoked responses during iontophoresis. Data were collected when changes in neural activity reached a steady state (typically within 5–10 min). The current levels used were not always those that produced a maximum effect. After the effects of the drug had been determined, drug release was terminated and the holding current was reapplied. Recovery was observed (usually after 10–15 min) by monitoring the waveform of spikes and the level of activity after the drug release was discontinued.

**Control experiments**

To exclude the possibility that iontophoretic current contributed to the change of response during drug application, control experiments were conducted. After TEA was applied to a cell and a change of response and/or spontaneous firing was observed, the cell was allowed to recover fully. Then the same amount of current as used for the release of TEA was applied to the barrel with saline alone. Spontaneous firing and responses to tone bursts and AM tones were then recorded again.

In our study, 4 neurons were studied using this procedure. For each of these neurons, no change in response and/or spontaneous firing was observed when current was applied to saline alone, although changes did occur during iontophoresis of TEA.

**Data analysis**

The effect of TEA on the responses to tone bursts was studied by comparing the number of spikes recorded before and during drug application. For this purpose, spikes generated within a time window of 120 ms from the onset of each tone burst were counted. The number
of spikes recorded at different SPLs was used for the generation of a rate-level function. The rate-level functions obtained before and during drug application were then compared. The effect of TEA on the responses to tone bursts was also studied by comparing the shapes of poststimulus time histograms (PSTHs) before and during drug application.

To study the effect of TEA on spontaneous activity, the number of spikes within a time window of 500 ms immediately before the onset of a tone burst was counted and the firing rate was determined. The effect of TEA on the responses to AM tones was studied by comparing the firing rate and response synchrony before and during drug application. Spikes recorded during the first 120 ms of each stimulus presentation were excluded from the analysis to avoid contamination by onset responses. Therefore the firing rate was calculated based on the number of spikes after the first 120 ms until the end of the stimulus. Two neurons that responded only to the onset of the AM stimulus could not be subjected to this analysis. Firing rate was plotted versus modulation frequency to create a firing rate modulation transfer function (MTF FR ). In the present study, the profiles of MTF FR were broadly classified into 3 groups: band-pass (modulation transfer functions with a single peak), band-suppress (those with a trough), and others. The classification was based on our previous observations that neurons with band-pass (53%) and band-suppress (25%) firing patterns are the two major groups in the ICC (Zhang and Kelly 2003).

To study response synchrony, the occurrence time of each spike was determined with reference to the onset of the modulation cycle within which the spike was elicited, and a modulation period histogram was created. A measure of vector strength, as described by Goldberg and Brown (1969), was used to characterize the synchrony of neural firing with regard to the modulation envelope. Vector strength (VS) was defined as

\[ VS = \frac{\sqrt{\sum \sin(a_i)}}{N} \]

where \( a_i \) is the phase of spike \( i \) relative to the sinusoidal modulation cycle [i.e., \( 2\pi \cdot \text{frequency of modulation} \cdot \text{occurrence time of spike} \cdot i \)]. A VS of 1 indicates perfect phase-locking, whereas a VS of 0 reflects a lack of correlation between the neural firing and the modulation envelope. Vector strength was plotted versus modulation frequency to create a temporal modulation transfer function (MTF VS ).

**RESULTS**

In this study, we recorded spikes extracellularly from 61 single neurons in the rat’s ICC. The effect of TEA on spontaneous firing and responses to tone bursts was examined in the entire population of neurons, whereas the drug effect on responses to AM tones was examined in 46 neurons.

**Effect of TEA on the spike waveform**

Release of TEA increased the width of spikes, as shown in Fig. 1. For a typical spike with both a positive and a negative phase, the widening was more pronounced for the negative (2nd) phase than the positive (1st) phase. The widening of spikes was observed in all neurons during application of TEA.

**Effect of TEA on spontaneous firing**

Forty-three of the 61 neurons examined in this study showed a significant increase in spontaneous firing with TEA (based on a z-score criterion for an increase in activity in each neuron, \( P < 0.01 \)). The average spontaneous firing rate for the 61 neurons examined was 3.3 spikes/s before drug application, compared with 17.7 spikes/s during TEA. Statistical analysis revealed that the spontaneous firing rate was significantly increased in the population of 61 neurons during TEA (\( z = 6.566, P < 0.005 \), sign test).

An example of the increase in spontaneous activity is shown for a representative neuron in Fig. 2. Before drug application this neuron had a low spontaneous firing rate (Fig. 2A, 1st row). The spontaneous activity greatly increased during application of TEA (Fig. 2A, 2nd row) and became low again after cessation of drug application (Fig. 2A, 3rd row). The average spontaneous firing rate before, during, and after application of TEA is shown in Fig. 2C.

**Effect of TEA on the responses to tone bursts**

The effect of TEA on tonal responses was determined for the entire population of 61 neurons. Using a change of 20% in the number of spikes at 10 dB above threshold as a criterion, we found that TEA increased response strength in 37 neurons and decreased response strength in 9 neurons. The drug did not change responses in the remaining 15 neurons. Although changes in the temporal features of the PSTH were observed in some ICC neurons during TEA, these changes were neither prominent nor systematic. Neurons with sustained response patterns (55 cells) still showed a sustained pattern of firing and those with onset patterns (6 cells) still showed an onset response pattern during TEA.

Figure 2 shows an example of the increase in tone-evoked responses during application of TEA. Before drug application, this neuron showed sustained firing in response to a tone burst (Fig. 2A, 1st row). The number of spikes was greater at higher stimulus intensities over the range between 20 and 80 dB SPL. The rate-level function had a monotonic shape (Fig. 2B). TEA enhanced the response to tone bursts over the entire range of suprathreshold stimulus intensities. However, the basic shape of the rate-level function remained the same. The effect of TEA on tonal responses was reversible. When the drug was discontinued, response strength returned to the predrug level as indicated by the rate-level function shown in Fig. 2B.

For the neuron presented in Fig. 2, the increase in firing rate to tone bursts at 10 dB above minimum threshold was from 19.2 to 79.6 spikes/s. The increase in spontaneous firing was from zero to 16.4 spikes/s. The shape of the PSTH was basically unchanged during TEA (Fig. 2A, 2nd row), in spite of the
increase in the number of spikes. Although the tonal responses and spontaneous activity increased during TEA, firing remained low at the offset of a tone burst.

Figure 3 shows a neuron with a pause in the PSTH between the onset and sustained parts of its response to tone bursts before drug application (Fig. 3A, 1st row). For this neuron the firing rate was also increased by TEA. Before the drug, the response strength increased from slightly above 0 spikes/trial at 30 dB SPL to about 4 spikes/trial at 40 dB SPL and remained at this saturated level with further increases in stimulus intensity (Fig. 3B). The spontaneous firing rate was low (Fig. 3C). TEA increased the response over the entire range of stimulus intensities. The increase was more apparent at 40 dB SPL than at other intensities, yielding a nonmonotonic rate-level function (Fig. 3B). The increase in responses to tone bursts (by 32.1 spikes/s at 10 dB above threshold at CF) was accompanied by an increase in spontaneous firing (by 40.3 spikes/s). The basic shape of the PSTH was unchanged during drug application (Fig. 3A, 2nd row). Specifically, the pause between the onset and the sustained part of the response was still present. Similar to the result shown in Fig. 2, firing remained low at the offset of the tone burst during drug application in spite of an increase in both tone-evoked responses and spontaneous activity. When TEA was discontinued, both response strength (Fig. 3B) and spontaneous firing rate (Fig. 3C) returned to predrug levels.

TEA reduced responses to tone bursts in 15% of the ICC neurons, as illustrated in Fig. 4 by a cell with an onset firing pattern (Fig. 4A, 1st row). Before the drug, the response increased with stimulus intensity and reached a saturation point at 40 dB SPL. Little spontaneous firing was observed. During application of TEA, the strength of the response was reduced at all suprathreshold intensities (Fig. 4B), in spite of a significant increase in spontaneous firing (z-score, P < 0.01; Fig. 4A, 2nd row and Fig. 4C). The reduction of response strength was more pronounced at higher stimulus intensities (Fig. 4B). The onset response pattern was unchanged during TEA. The predrug rate-level function was restored after drug application was discontinued (Fig. 4B).

We compared the effect of TEA on tonal responses and spontaneous activity for every neuron in our sample. The data from all neuron are plotted in Fig. 5 with the abscissa showing the change of firing rate to a tone burst at 10 dB above threshold and the ordinate showing the change of spontaneous firing. Data points located above the horizontal axis represent an increase in spontaneous firing, and those located to the right of the vertical axis represent an increase in tone-evoked activity. The distribution of data shows that TEA increased spontaneous activity and changed (either increased or decreased) responses to tone bursts for most ICC neurons. Most data points in Fig. 5 are located off the diagonal line, indicating that the magnitude of change in tone-evoked responses was usually different from that in spontaneous firing. Statistical analysis showed that the drug-induced change in spontaneous firing rate and the change in tonal response were not highly correlated (r = 0.37).
We first present data for 26 neurons with a band-pass MTF\textsubscript{FR}. As shown by the 3 examples in Fig. 6, most neurons in this group increased their responses to AM tones during TEA. For the neuron in Fig. 6A, the increase in firing rate was much larger at off-peak modulation frequencies than at the peak modulation frequency. As a result, the MTF\textsubscript{FR} lost its peak and became almost flat during TEA. When the drug was discontinued, the MTF\textsubscript{FR} returned to its predrug shape. For the neuron in Fig. 6B, the MTF\textsubscript{FR} also became almost flat during TEA, although a peak could still be seen. For both neurons in Fig. 6, A and B, TEA reduced the selectivity of the response to the modulation frequency.

For the neuron shown in Fig. 6C, TEA resulted in a constant increase in firing rate across all modulation frequencies. The shape of the predrug MTF\textsubscript{FR} was unchanged, indicating that frequency selectivity was not reduced during TEA.

For each of the neurons shown in Fig. 6, there was an increase in the spontaneous firing rate as indicated by the bar graphs (Fig. 6, A–C, right panels).

Twelve of the 26 neurons with a band-pass MTF\textsubscript{FR} before drug application showed a broadening or flattening of the MTF\textsubscript{FR} curve during TEA, indicating a reduction in frequency selectivity. Six neurons showed a nearly constant upward shift in firing rate across modulation frequencies and retained the shape of their MTF\textsubscript{FR}. Five neurons showed other types of change during application of TEA, whereas 3 neurons showed no noticeable changes.

For 11 of 18 neurons with band-suppressed or other types of MTF\textsubscript{FR}, the firing rate in response to AM tones was increased by TEA for either all or some part of the range of modulation frequencies. Shown in Fig. 7, A and B are two neurons with a band-suppressed MTF\textsubscript{FR}. For the neuron in Fig. 7A, the increase in firing rate produced by TEA was greatest when the modulation frequency was lower than 20 Hz or higher than 100 Hz. There was relatively little change when the modulation frequency was between 20 and 100 Hz. Therefore the band-suppressed feature of MTF\textsubscript{FR} was enhanced during TEA. In contrast, the neuron shown in Fig. 7B displayed a roughly constant increase in firing rate across modulation frequency during application of TEA. As a result, the MTF\textsubscript{FR} for this neuron underwent an overall upward shift without a major change in the shape.

The neuron in Fig. 7C showed a constant level of firing across a wide range of modulation frequencies (i.e., an all-pass MTF\textsubscript{FR}) before TEA. Application of the drug resulted in an overall upward shift in activity without a substantial change in the shape of the MTF\textsubscript{FR} profile.

Each of the neurons shown in Fig. 7 increased its spontaneous firing rate during application of TEA as indicated by the bar graphs (Fig. 7, A–C, right panels).

We determined the effect of TEA on firing rate for 44 neurons with steady firing in response to AM tones, including...
the 26 with band-pass and the 18 with band-suppressed or other types of MTF FR. TEA increased responses to AM tones for 29 neurons, reduced responses (over either the entire range or a part of the range of modulation frequencies) for 7 neurons, and did not cause a noticeable change in firing rate in the remaining 8 neurons.

**Effect of TEA on the synchrony of responses to AM tones**

The effect of TEA on the synchrony of responses to AM tones was studied by comparing the MTF VS before and during drug application. Results from a typical neuron are shown in Fig. 8. Before drug application, this neuron showed a high level of synchrony when the modulation frequency was below 200 Hz. The MTF VS had a band-pass shape and peaked at 60 Hz with a vector strength of 0.95 (Fig. 8A). The MTF FR also had a band-pass shape and peaked at 40 Hz (Fig. 8B). During TEA vector strength was greatly reduced when the modulation frequency was below 100 Hz (Fig. 8A). Responses at all modulation frequencies (Fig. 8B) and the level of spontaneous firing (Fig. 8C) were increased.

TEA reduced vector strength in 22 out of 33 neurons that showed a single peak in their MTF VS (band-pass type) before TEA. Eleven of these neurons showed a reduction mainly at the low frequency flank of their MTF VS, 4 neurons showed a reduction at both upper and lower flanks, 5 neurons showed a reduction mainly at the peak and low frequency flank, and 2 neurons showed a reduction at all modulation frequencies. For all 33 neurons, vector strengths obtained before and during TEA were compared at the peak modulation frequency, half an octave below the peak modulation frequency, one octave below the peak modulation frequency, and half an octave above the peak modulation frequency. The reduction in vector strength at each of these modulation frequencies was statisti-
cally significant (Table 1). For the 11 neurons not showing a band-pass MTFVs, but whose vector strength could be reliably calculated, TEA reduced the vector strength in 6 neurons and caused complex changes in 3 neurons. The drug did not change vector strength in the remaining 2 neurons.

The shape of the period histogram was also examined to determine the effect of TEA on response synchrony. For the neuron shown in Fig. 8, spikes were distributed over a limited portion of the modulation period before drug application. During TEA, spikes became more broadly distributed over the modulation cycle. The expansion of spike distribution over the modulation period was especially pronounced at 10 and 60 Hz, resulting in a large reduction of vector strength at these frequencies.

A reduction in vector strength was usually accompanied by an increase in firing to the AM stimulus as shown in Fig. 8. For the 28 neurons that showed a reduction in vector strength during TEA, 22 of them showed an increase in firing rate. The other 6 neurons showed a reduction or no change in firing during TEA. In these 6 cases, the reduction in vector strength was attributed to complex changes in the spike distribution over the modulation cycle. For example, Fig. 9 shows a neuron with a band-pass MTFVs that peaked at 60 to 100 Hz. TEA reduced vector strength at off-peak modulation frequencies (Fig. 9A); however, it did not produce any consistent increase or decrease in the firing rate (Fig. 9B). The reduction in vector strength was most apparent at 40 Hz (Fig. 9A), although there was no change in firing rate at this frequency (Fig. 9B). However, there was a change in the distribution of spikes at 40 Hz (Fig. 9D, 2nd row).

The change in vector strength was not highly correlated with a change in spontaneous activity. The correlation coefficient r between the change in spontaneous activity and the change in vector strength at best modulation frequency (BMF) was only 0.212398. The values of r at frequencies 0.5 octave above or 0.5 octave below BMF were 0.23072 and 0.319141, respectively. The r at the modulation frequency that produced the greatest change in vector strength was only 0.17667; thus an increase in spontaneous activity did not account for the changes in response synchrony.

**DISCUSSION**

The role of potassium channels in generating neural responses to sounds is a largely unexplored area. By using a nonspecific potassium channel blocker TEA we revealed that
potassium channels are important for regulating the level of spontaneous activity as well as the strength and temporal pattern of neural responses to acoustic stimuli in the ICC.

Repolarization and excitability

Application of TEA produced a prominent and consistent widening of the spike waveform. For a typical spike with 2 phases, the widening was more apparent in the 2nd phase, a change that would be expected from a block of the delayed rectifier responsible for repolarization of the action potential (Hille 2001). Block of other TEA-sensitive potassium channels, such as the large conductance (BK-type) calcium-activated potassium channel or the M-type potassium channel, might also have contributed to the change in spike waveform (Nicholls et al. 2001).

The effect of TEA on the excitability of ICC neurons could be attributable to a disruption of any one of several TEA-sensitive potassium channels responsible for maintenance of the resting potential [e.g., the BK-type calcium-activated potassium channel, the M-type potassium channel or the low threshold potassium channel (Hille 2001; Nicholls et al. 2001)]. Block of these channels would be expected to shift the membrane potential upward and enhance neural excitability (Agar et al. 1996; Fu et al. 1996; Hirsh and Oertel 1988; Nicholls et al. 2001), which could, in turn, result in an increase in spontaneous activity and/or sound-evoked responses. In our study, an increase in spontaneous and evoked activity was, indeed, observed in the majority of the ICC neurons during application of TEA.

Brain slice studies of neurons in the cochlear nucleus and the superior olivary complex have shown that low-threshold potassium channels reduce excitability and suppress responses to current pulse stimulation in vitro (Brew and Forsythe 1995; Manis and Marx 1991; Rathouz and Trussell 1998; Reyes et al. 1994; Svirskis et al. 2002), suggesting the possibility that these channels play a role in the maintenance and regulation of spontaneous and/or evoked activity in vivo at least in some auditory structures. Further data are needed, however, to determine whether low-threshold potassium channels might contribute to neural excitability in the ICC or whether other potassium channels might be involved.

For some neurons there was a reduction in sound-evoked responses with TEA. This reduction might have resulted from a change in synaptic input due to a block of presynaptic potassium channels. Pharmacological block of these potassium...
channels could have widened spikes and enhanced transmitter release (Hille 2001), resulting in greater excitation or inhibition depending on the transmitter released and the nature of the postsynaptic receptor. Moreover, the drug might have affected the excitability of neurons in local circuits within the ICC (González-Hernández et al. 1996; Moore et al. 1998; Yang et al. 2000), resulting in an increase in the number of spikes that impinge on the neuron from which recordings are made. Because synaptic inputs might be either excitatory or inhibitory, the result could be either an increase or a decrease in neural activity. This may explain why some neurons show an increase and others a decrease in activity during application of TEA.

**TABLE 1. Change of vector strength**

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 Octave Below BMF</th>
<th>0.5 Octave Below BMF</th>
<th>BMF</th>
<th>0.5 Octave Above BMF</th>
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<tr>
<td>Average vector strength before drug</td>
<td>0.59</td>
<td>0.68</td>
<td>0.89</td>
<td>0.57</td>
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<tr>
<td>Average vector strength during TEA</td>
<td>0.47</td>
<td>0.54</td>
<td>0.79</td>
<td>0.52</td>
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<tr>
<td>df</td>
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<td>32</td>
<td>33</td>
<td>22</td>
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<td>5</td>
<td>2.29</td>
</tr>
<tr>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Potassium channels and processing of amplitude-modulated sounds**

AM is an important aspect of acoustic stimulation. Sinusoidal AM tones have been widely used to study how neurons at successive levels of the ascending auditory pathway process temporal properties of sound (for review, see Frisina 2001; Langner 1992). Compared with lower auditory nuclei, more neurons in the ICC show band-pass MTFR and high maximum vector strength in response to AM tones as shown by Krishna and Semple (2000) and Zhang and Kelly (2003) for the anesthetized gerbil and rat, respectively (the anesthetics included pentobarbital and ketamine/xylazine). The band-pass profile of MTFR provides a potential place code for modulation rate and the high maximum response synchrony provides a possible temporal code. Attempts have been made to investigate how synaptic receptors in the ICC shape response characteristics to AM tones (Burger and Pollak 1998; Caspary et al. 2002; Zhang and Kelly 2003). It has been found that the local AMPA, NMDA, and GABA receptors are involved in the regulation of the overall level of response. However, these receptors are not likely responsible for specific profiles of MTFR (e.g., band-pass) or maximum level of synchronization in the ICC (Zhang and Kelly 2003).

In the present study we found that TEA changed the shape of MTFR in the ICC. Specifically, for some neurons with a band-pass MTFR, TEA widened or even completely flattened
the curve. TEA also reduced vector strength for many ICC neurons. Our results suggest that TEA-sensitive potassium channels are important for regulating the selectivity of responses for specific modulation rates.

A non-specific increase in firing and the resulting change in the MTF_{FR} are probably related to a depolarizing shift in the membrane potential. More specific changes, however, might be related to the temporal dynamics of potassium channels that are sensitive to TEA. Sinusoidal AM tones would be expected to activate potassium channels in ICC neurons periodically. Because each type of channel has unique temporal dynamics for activation, different effects might be produced by TEA at different modulation frequencies depending on the types of potassium channels involved and the extent of their involvement. These different effects may result in changes in the MTF_{FR} profile.

TEA may also change the MTF_{FR} profile by affecting neuronal intrinsic oscillation. In vivo patch-clamp recordings from neurons in the bat’s ICC have revealed oscillating currents that persist after tone burst stimulation (Covey et al. 1996). Intrinsic oscillation may facilitate responses to rhythmic stimuli with a matching frequency component, resulting in a peak in MTF_{FR}.

Studies have also revealed that the frequency of intrinsic oscillation can be determined by the combination of specific potassium and other ion channels (Hutcheon and Yarom 2000; Llinas 1988). A study of the inferior olivary nucleus showed that the coupling between calcium-dependent potassium channels and calcium channels caused rhythmic activity (Lang et al. 1997). Large-conductance calcium-dependent potassium channels are present in ICC neurons that exhibit transient spike discharges to positive intracellular current injections (Sivaramakrishnan and Oliver 2001), and they are sensitive to TEA. Therefore this subtype is a possible candidate involved in intrinsic oscillation and regulation of the MTF_{FR} profiles of ICC neurons.

The effect of TEA on response synchrony may also be related to the block of specific potassium channels. Brain slice and computational studies of lower auditory brain stem structures suggest that fast acting low-threshold potassium channels contribute to the high response synchrony of auditory neurons (Reyes et al. 1996; Rothman and Young 1996). A recent study of the gerbil medial superior olive showed that low-threshold potassium channels ensured high response synchrony when a cell received periodically modulated weak random inputs.
The high-threshold potassium channel expressed by gene Kv3.1 enhances the high-frequency synchronization in the ICC. For example, a type of high-threshold potassium channel expressed by gene Kv3.1 exists in the inferior colliculus (Perney et al. 1992; Wang et al. 1998). Neurons with this type of potassium channel have narrow action potentials and are able to fire at very high frequencies. These characteristics would allow neurons to follow AM stimuli with a high level of response synchronization. Genes encoding Kv9.1 and Kv2.1 channels have also been found in the rat’s IC (Richardson and Kaczmarek 2000). The coexpression of Kv9.1 and Kv2.1 shapes the temporal firing patterns of neurons. The channels expressed by genes Kv3.1 and Kv2.1/Kv9.1 are subject to block by TEA. Therefore the reduction in response synchrony during TEA may be attributable to its action on these channels.

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